Separation of the Phosphorus Compounds Present in the Acid-Soluble Fraction of Bovine Retina

Once the function of phosphorus in muscular work had been established, the question arose whether the phosphorus compounds have some function in vision as well, since, for its production, the energy from phosphorus compounds, which develops rapidly, seemed very suitable.

Nevertheless, although a great number of works have been dedicated to the solution of the problem and have succeeded in confirming the presence in the retina of a considerable quantity of organic and inorganic phosphorus, so far no intensive efforts have been made to isolate the various phosphorus compounds present in the acid-soluble fractions of the retina, if exception be made of the data of HOARE and KERLY[1] and of VENKSTERN[2]. In my previous researches[3], I have been able to demonstrate that, in the retina, the phosphorus compounds with a high content of energy prevail.

The data obtained by means of the effect dialysis and of activators and inhibitors (KERLY and BOURNE[4], SÜLLMANN and VOSS[5]) have permitted the conclusion that, for the retina also, the formation of lactic acid follows the same route of phosphoric glycolysis as in extracts of muscle; nevertheless the intermediate phosphoric esters have not been isolated.

There are few data on these important compounds: I have therefore thought it of particular interest to carry out a systematic research with the special aim of elaborating an analytical method that would avoid those inconveniences that inevitable occur whenever an attempt is made to apply the classical technique of LE PAGE[6] to the retina.

Experimental part.—Bovine retinas removed from the eyeballs immediately after slaughter of the animal and frozen in dry ice are submitted to cold extraction with 6 volumes of 3% perchloric acid. The acid filtrate is treated with a mixture of equal parts of Norite-celite, 200 mg per 5 ml of the extract, to get rid of the nucleotides. These are then eluated from the Norite-celite with 10% aqueous pyridine. The pyridine extract is then lyophilized and preserved for the chromatographic analysis. The filtrate, after treatment with Norite-celite, is treated with ammonium molybdate to remove the free as well as the creatinic phosphorus. The excess of molybdate is precipitated by H_2S. After removal of the excess of H_2S by bubbling air through, the phosphoric esters remaining are precipitated as salts of barium in excess of alcohol, then resuspended in H_2O, and treated with Amberlite (IR-120 H). The liquid is lyophilized and preserved for chromatographic analysis. This is performed both for the phosphoric esters and for the nucleotides, using the unidirectional ascending technique at 22°C. The strips were Whatman paper No. 1 (35 × 18) and the solvent used was made up of 15 ml of 30% trichloroacetic acid, 25 of 80% formic acid, 40 of normal butanol, 20 of normal propanol, 25 acetone.

[1] D. S. HOARE and M. KERLY, Biochem. J. *58*, 38 (1954).
[2] T. V. VENKSTERN, *Uspei sovremennoy Biology*.
[3] E. DE BERARDINIS and G. AURICCHIO, Ann. oft. Cl. ocul. *77*, 1 (1951); *78*, 53 (1952).
[4] M. KERLY and M. BOURNE, Biochem. J. *34*, 563 (1940).
[5] H. SÜLLMANN and T. A. VOSS, Enzymologia *6*, 246 (1939).
[6] J. A. LE PAGE, *Manometric Techniques and Tissue Metabolism* (N. W. Umbreit and R. H. Burris, Burgess Publ. 1949).

	Determined	Calculated
Inorganic phosphorus P_i	25·8	—
Creatinic phosphorus P_c	11·4	11·0
Fraction 1 phosphorus	5·0	10·8
Fraction 2 phosphorus	7·1	—
Total extract phosphorus	48·0	49·3

To demonstrate the phosphorus compounds on the chromatograms, the technique of BANDURSKI and AXELROD[7] was used. In the Table, the relatives values in the distribution of the phosphorus in the various fractions are reported. The values are expressed in mg of P/100 g of fresh retina and represent the mean values of several determinations.

In the first column the values of the phosphorus determined experimentally are given, while the second column contains the values determined indirectly, that is calculated. The P_c was in fact determined both directly after precipitation of the P_i and indirectly as the difference between free inorganic P and 'true' inorganic P, that is P_i. Thus the fractions 1–2 were determined both directly after incineration and by the difference between the total P and $P_i + P_c$.

The total P calculated on the basis of single values obtained experimentally (see column I) differs somewhat (2·5%) from that determined in the extract, which is probably due to the fact that, in the fractions 1–2, free phosphorus was still present to some small degree.

Further data and considerations will be given when the work is presented *in extenso*.

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Riassunto

L'autore ha studiato i composti fosforici della retina interessati nella glicolisi, mediante una tecnica combinata chimica e cromatografica. Tale tecnica ovvia agli inconvenienti legati alle classiche tecniche di LE PAGE ed HANES e HISHERWOOD. I risultati ottenuti confermano l'esistenza nella retina di un attivo metabolismo glicidico che segue la via normale senza pertanto escludere una possibile via collaterale.

[7] R.S. BANDURSKI and B. AXELROD, J. biol. Chem. *193*, 405 (1951).

Studio comparativo degli aminoacidi presenti negli estratti e negli idrolizzati di retina e di epitelio corneale di bue

L'interesse sempre crescente per lo studio della distribuzione degli aminoacidi liberi, quale spia del ricambio proteico in un dato tessuto, mi ha indotto ad affrontare questo genere di studio nel caso della retina e dell'epitelio corneale. Ho pertanto stabilito il quadro normale degli aminoacidi liberi in questi due tessuti, nonché il numero ed il tipo di aminoacidi presenti negli idrolizzati di questi tessuti, essendo la letteratura al riguardo scarsa e frammentaria[1].

[1] A. J. SCHAEFFER, Docum. ophtal. *5*, 403 (1951).