Letters to the Editor

The Editor does not hold himself responsible for opinions expressed by his correspondents. He cannot undertake to return, or to correspond with the writers of, rejected manuscripts intended for this or any other part of NATURE. No notice is taken of anonymous communications.

NOTES ON POINTS IN SOME OF THIS WEEK'S LETTERS APPEAR ON P. 879.

CORRESPONDENTS ARE INVITED TO ATTACH SIMILAR SUMMARIES TO THEIR COMMUNICATIONS.

Formation of a New Phosphate Ester in Kidney Extracts

THE phosphorylation of sugars and glycerol in kidney extracts is increased by addition of fumaric or malic acid, and this effect is caused by a stimulation of the tissue respiration¹.

If fumaric or malic acid is added to extracts from washed kidney pulp, the phosphorylation, which is very slight in preparations without substrate, is increased considerably and the respiratory rate is also accelerated. Since these extracts are very poor in phosphate acceptors, it is most likely that fumarate and malate are converted through oxidation to a phosphate acceptor. A more detailed investigation definitely established the formation of a new phosphoric ester quite different from the hexosediphosphate formed from glucose and fructose². The characteristics of the phosphoric ester formed from the oxidation products of malate agree completely with those of phosphopyruvic acid³.

Kidney cortex from a fasting rabbit was minced and washed twice with two volumes of ice water. The washed tissue was then ground with sand and extracted with an equal volume of *M*/15 sec. phosphate which contained 0.2 per cent fluoride. Aliquot parts (1.5 c.c.) were incubated in an oxygen atmosphere at 37° for 30 min.

The phosphate analyses were calculated to mgm. P in the entire extract.

	O ₂ uptake	Pdir.	Palk.	Piod.	Pmerc.	P ₃₀
Control (initial sample)		1.16	1.17	1.18		1.16
Incub. without substrate	474	1.00	1.00	1.02		1.11
Incub. with glucose, 40 mgm.	323	0.75	0.83	0.77	0.75	0.96
Incub. with malate, 15 mgm.	720	0.78	0.78	0.98	1.00	1.12

Pdir. : phosphate determined directly.

Palk. : phosphate after 20 min. incubation in *N*/1 caustic soda.

Piod. : phosphate after 20 min. incubation in *N*/10 alkaline iodine.

Pmerc. : phosphate after 10 min. incubation in neutral mercuric chloride.

P₃₀ : phosphate after 30 min. hydrolysis in *N*/1 hydrochloric acid at 100°.

The ester derived from malic acid has not been isolated, but since it is hydrolysed by mercuric chloride, which is a specific reagent for the hydrolysis of acetal esters, it is probably the phosphoric ester of the enolic form of pyruvic acid. The phosphopyruvic acid could not originate from traces of sugars because fluoride inhibits the formation of this compound from phosphoglyceric acid. The esterification product, however, contains no phosphoglyceric acid, since the P₃₀ value indicates that no acid resistant ester is formed from malate. It therefore appears

that the phosphopyruvic acid had its origin from oxalacetic or pyruvic acid derived from the oxidation of malic acid. Malate is converted to carbohydrate in the kidney cortex⁴, and it appears from these facts that phosphopyruvic acid is an intermediate in the formation of sugars from malate and lactate (cf. Green⁵, Meyerhof⁶).

HERMAN KALCKAR.

Institute of Medical Physiology,
University of Copenhagen.

Sept. 22.

¹ Kalckar, XVI Internat. Congr. of Physiology, Zurich (1938).

² Kalckar, *Enzymologia*, 2, 47 (1937).

³ Meyerhof and Lohmann, *Biochem. Z.*, 273, 60 (1934).

⁴ Benoy, M., Baker, Z., and Elliott, K., *Biochem. J.*, 31, 1268 (1937).

⁵ Green, Needham and Dewan, *Biochem. J.*, 31, 2327 (1937).

⁶ Meyerhof, Ohlmeyer and Möhle, *Biochem. Z.*, 297, 90 (1938).

Diffusion of Phosphate Ions into Blood Corpuscles

WE find on shaking labelled (radioactive) sodium phosphate of negligible weight with blood, that phosphate ions quickly enter the erythrocytes. In the course of three hours, nearly half the phosphate ions present in the plasma of rabbit's blood diffuses into the corpuscles. When labelled phosphate ion enters the cell, it becomes available for esterification processes. Simultaneously, non-labelled phosphoric acid-ester molecules decompose, producing non-labelled phosphate which diffuses into the plasma; this process is going on incessantly. There is thus a rapid interchange of phosphorus atoms between plasma and cells. In *in vivo* experiments, using a newly developed technique¹, we find the same rate of penetration of phosphate ions into corpuscles as occurs *in vitro*. In both cases, not all the acid-soluble organic phosphorus compounds exchange their phosphorus for the labelled variety, and it is of interest to note that a large part of the phosphorus esters which resist hydrolysis when heated for an hour at 100° with mineral acids do not become labelled in the course of a few hours.

In order to compare the rate of diffusion of the phosphate ion and that of ester molecules, in some of our experiments we added labelled hexosemonophosphate (kindly presented to us by Prof. Parnas) to blood. We find that if hexosemonophosphate diffuses at all into cells, the rate is at least ten times less than that of phosphate ions. In *in vitro* experiments, labelled hexosemonophosphate was found to be to a large extent decomposed, presumably by Robison's bone phosphatase present in blood. The rate of hydrolysis is that of a monomolecular reaction, the velocity constant being 0.0024 min.⁻¹. The enzymatic decomposition of the ester also takes place in the absence of corpuscles. In the intact animal, the blood comes in rapid and intimate contact with the large surface of bone tissue containing the