XVII. THE PHOSPHORIC-ESTERASE OF BLOOD AT VARIOUS HYDROGEN ION CONCENTRATIONS.

By MARJORIE MARTLAND.

From the Biochemical Department, the Lister Institute.

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IN a recent paper [Martland, Hansman and Robison, 1924] it was shown that when defibrinated blood is kept for 2–3 hours at 38° in the presence of small quantities of added acid, or in an atmosphere containing excess of CO₂, the concentration of inorganic phosphate is increased owing to enzymic hydrolysis of the phosphoric esters present in the blood. Conversely, additions of small amounts of alkali, or ventilation of the blood with CO₂-free air, gave rise under similar conditions to a synthesis of phosphoric ester with reduction in the amount of free phosphate, an observation which has been made independently by Lawaczeck [1924].

From these experiments it was suspected that the regulating mechanism which determines the activity of the esterase might prove to be the hydrogen ion concentration of the blood, but accurate estimations of p_H were not at that time made. The object of the present work was to make a closer study of this relationship, and to determine whether such changes in reaction as might be found to affect the equilibrium between the hydrolytic and synthetic activities of the enzyme are those which might occur in the blood of the living organism.

EXPERIMENTAL.

A method was devised by which blood at 38° might be kept at a stable p_H for a length of time sufficient for the study of the action of the phosphoric esterase. To the defibrinated whole blood of a rabbit $N/2$ sulphuric acid was added, drop by drop, in a measured amount sufficient to neutralise the plasma bicarbonate and yet insufficient for the production of acid haematin. The blood and acid were mixed and allowed to stand at room temperature for 5 minutes, to allow the hydrogen ion concentration within the corpuscles to come into equilibrium with that of the plasma, at the end of which time samples were withdrawn for estimation of p_H and of inorganic phosphate. The blood, contained in a large flask, was then kept at 38° for half an hour, during the whole of which time the flask was rotated rapidly so as to spread the blood in a thin film over the wall of the vessel, the air of the room being drawn over the blood at 5 minute intervals. By this means the CO₂ tension

of the blood was brought into equilibrium with that of the surrounding atmosphere.

Samples having been withdrawn at this point for estimation of p_H and free phosphate, the blood was divided into aliquot parts and different amounts of $N/2$ caustic soda added to each. The suitable quantities of alkali had been found empirically after a considerable number of experiments, the object being to bring the p_H of the blood to values lying closely on either side of the point at which synthesis and hydrolysis are in equilibrium. The p_H and inorganic phosphate were estimated 5 minutes after adding the alkali and again after standing, without shaking, at 38° for periods varying from half-an-hour to one-and-a-half hours. No experiment was prolonged beyond two hours from the killing of the animal, it having been found that the synthetic activity of the enzyme became markedly less after that time.

Estimations of blood sugar were made parallel with the p_H and phosphate determinations, in view of the possibility that the phosphoric ester may be present in the blood in combination with a reducing group. The p_H was estimated by the method of Dale and Evans [1920], the inorganic phosphate by that of Briggs [1922], and the sugar by the method of Hagedorn and Jensen [1923].

The results of three experiments are here set forth, the phosphate and sugar being expressed as mg. per 100 cc. of defibrinated whole blood (D).

	Vol. of $N/2$ H_2SO_4 per 10 cc. D	Vol. of $N/2$ NaOH added after 30 minutes cc.	Time (mins. from beginning of experiment)	p_H	Sugar mg.	Inorganic phosphate mg. P	Hydrolysis (H) or Synthesis (S) of phosphoric ester mg. P
<i>Exp. 1.</i>							
0.7 cc.	—	}	5	<6.50	125	5.15	
			35	6.83	128	6.63	1.48 H.
0.7	0.3	}	40	7.13	127	6.62	
			95	7.16	134	8.51	1.89 H.
0.7	0.4	}	40	7.36	122	6.58	
			95	7.38	108	5.33	1.25 S.
0.7	0.5	}	40	7.48	123	6.62	
			95	7.51	92	5.22	1.40 S.
<i>Exp. 2.</i>							
0.7 cc.	—	}	5	<6.50	128	4.92	
			30	6.81	128	5.71	0.79 H.
0.7	0.3	}	35	7.15	126	5.53	
			80	7.19	130	6.25	0.72 H.
0.7	0.4	}	35	7.35	117	5.35	
			80	7.39	112	4.56	0.79 S.
0.7	0.5	}	35	7.50	129	5.33	
			80	7.55	112	4.49	0.84 S.
<i>Exp. 3.</i>							
0.7 cc.	—	}	5	<6.50	137	4.88	
			30	6.79	141	6.08	1.20 H.
0.7	0.3	}	35	7.0	134	6.07	
			60	7.09	140	6.27	0.83 H.
0.7	0.4	}	120	7.11	135	6.90	
			65	7.28	136	6.65	
0.7	0.5	}	120	7.29	133	6.96	0.31 H.
			65	7.39	141	6.06	
0.7	0.5		120	7.41	120	5.30	0.76 S.

These tables show a definite relationship between p_H and inorganic phosphate. The variations in the blood sugar shed no new light on previous observations. At a p_H higher than 7.3 synthesis of organic phosphate and glycolysis proceed simultaneously, but at a p_H lower than 7.3 where hydrolysis of organic phosphate takes place, the blood sugar either remains constant or shows an increase so small as to be of doubtful significance. There is no evidence of any simple and direct connection between the esterification of phosphoric acid and the synthesis of non-reducing compounds from sugar. The occurrence of glycolysis in alkalosis has been noted by various workers, and has been correlated with an increase in the lactic acid of the blood [Anrep and Cannan, 1924].

DISCUSSION OF RESULTS.

It will be seen that after the preliminary removal of CO_2 in the first half-hour, the p_H of a given blood sample shows only very slight change from the time at which the alkali was added to the end of the experiment, and it is legitimate to assume that the reaction has remained approximately constant throughout such a period. In Exp. 3, hydrolysis of phosphoric ester has taken place at a p_H of 7.09–7.11, as also at 7.28–7.29, while at 7.39–7.41 there is decrease of inorganic phosphate indicating synthesis of the ester. The other experiments give comparable results, hydrolysis of phosphoric ester being found at every p_H lower than 7.29, and synthesis above 7.35. The critical point at which hydrolytic and synthetic processes are in equilibrium appears to lie between 7.3 and 7.35. This corresponds closely to the mean average p_H found in normal blood by various workers. Cullen and Robinson [1923] give a mean of 7.345 in normal human subjects, while in normal rabbits the mean average p_H has been found to be 7.42 for arterial blood, 7.345 for venous blood [Hawkins, 1924].

A survey of recent literature brings to light a number of instances in which alterations in the p_H of the blood *in vivo* are associated with changes in the amount of inorganic phosphate similar to those found *in vitro* in these experiments. In the observations made on a human subject by Haldane, Wigglesworth and Woodrow [1924], the acidosis induced by breathing an atmosphere containing 6–7 % CO_2 was computed to correspond to a fall of 0.09 in arterial p_H , the inorganic phosphate of the blood showing an increase from 3.39 mg. to 4.82 mg. after one-and-a-half hours under these conditions. Conversely, in alkalosis induced by forced breathing maintained for long periods, causing a reduction of alveolar CO_2 to 12 mm. and a calculated rise of about 0.3 in arterial p_H , the free phosphate of the blood was diminished to 1.25 mg. or less per 100 cc.

Kay [1924], approaching the same subject from a different point of view, has shown that the acidosis produced by ingestion of ammonium chloride gives rise to an increase of inorganic phosphate accompanied by a decrease in the amount of that fraction of the phosphoric esters upon which the esterase

of the blood has been shown to act [Martland, Hansman and Robison, 1924].

The blood changes in ether anaesthesia are significant also of a close connection between increased hydrogen ion concentration and hydrolysis of organic phosphate. Van Slyke, Austin and Cullen [1922] have shown that a true acidosis occurs in this condition, and after 20 minutes' administration of ether a p_H as low as 7.07 has been recorded [Austin, Cullen, Gram and Robinson, 1924]. It has been noted that during a similar period of ether anaesthesia in rabbits the amount of inorganic phosphate is very strikingly increased [Martland and Robison, 1924].

In the terminal stages of nephritis accompanied by the so-called uraemic syndrome the association of acidosis with increase of inorganic phosphate in the blood is well established. A case is reported by Van Slyke [1921] in which Cullen, using an electrometric method, found a blood p_H as low as 6.95 in a man dying of advanced nephritis. The very marked rise in the free phosphate of the blood in such conditions has been observed by de Wesselow [1923], who attaches a grave clinical significance to this change in the phosphorus metabolism.

A relationship between acidosis and hydrolysis of organic phosphate is suggested by the work of Allan, Dickson and Markowitz [1924], who obtain a large increase of phosphates in the urine after subcutaneous injection of phloridzin in dogs. No estimations of the blood phosphates have been made in these experiments, but the amount of phosphorus eliminated is of such an order that it would be difficult to account for it without assuming an increase of inorganic phosphate in the blood. The blood p_H in phloridzinised dogs has been found by another observer to lie between 7.01 and 7.29 [Ets, 1924].

Recent work of Cavins [1924] gives rise to speculations as to whether the ketosis which occurs in carbohydrate starvation may not be concerned in a hydrolysis of phosphoric ester. In experiments on rachitic rats, Cavins has first depleted the serum of inorganic phosphate by feeding with a diet deficient in phosphate and in fat-soluble A. He has then, on fasting these animals, found an increase of free phosphate to an amount far in excess of the normal, this rise being accompanied by a cure of the rachitic condition. Control experiments on non-rachitic rats showed however no such marked hydrolysis of phosphoric ester on fasting, and it may be that factors other than the p_H of the blood have been brought into action. It would be of interest to discover whether a true "uncompensated" acidosis may not have been induced by the starvation of the rachitic animals, the alkali reserve of the normal rats having possibly been adequate to keep the blood p_H constant, or their store of glycogen having been sufficient to avert any extreme ketosis within the time-limit of the experiments.

In each of the conditions enumerated, acidosis goes hand in hand with an increase, alkalosis with a diminution, of free phosphate. It is impossible to avoid making the inference that these changes are of the same nature as those shown to occur *in vitro*, a supposition which emphasises the importance of further study of the phosphoric ester—esterase reaction of the blood.

SUMMARY.

In blood as a result of the activity of the phosphoric-esterase hydrolysis of the phosphoric ester was observed when the p_H fell below 7.3, synthesis at a p_H above 7.35.

This relationship is suggested as an explanation of certain changes in the phosphate content of the blood associated with acidosis and alkalosis, which occur in diverse conditions in the living organism.

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