CLIX. PHOSPHATE AND CREATINE METABOLISM IN FEVER.

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WHILE making some observations in a case of juvenile G.P.I. undergoing malaria treatment it was noted that some urine specimens were practically devoid of phosphate. A search through recent text books and articles failing to give any reference to this phenomenon it was decided to study the problem further.

METHOD.

When the children had been infected with malaria by mosquitoes there was an incubation period of from 10–21 days. During this period observations on the blood and urine were made. The total urinary output was collected in three daily periods 7 a.m.–1 p.m., 1 p.m.–7 p.m., 7 p.m.–7 a.m. (the times arranged to suit the convenience of nursing), and the various estimations performed daily. This was continued until the pyrexia developed. From this point the whole of every specimen of urine passed was collected separately by the nurse and the time noted. Blood examinations were made at suitable times. This was continued until the attack was terminated by quinine administration. The collection of urine was continued until more or less normal figures were obtained. A further blood examination was then made. In no single case however was it possible for the complete programme to be carried out.

The analytical methods used were: urea—McLean; phosphate (blood and urine)—Briggs; creatine and creatinine (blood and urine)—Folin; blood sugar—micro-Folin-Wu. By simple adaptations of the blood methods it was found possible to do most of the investigations on about 1–1.5 ml. blood which was obtained from a single puncture of a finger. It was found that this disturbed the children far less than puncture of a vein. Even so it was not considered advisable to take as frequent specimens as could have been desired.

RESULTS.

Urine. Two sets of changes were observed—a gradual alteration of the total daily output of phosphate and creatine as the malaria infection increased in intensity and a rapid alteration during the pyrexial bouts. These changes are shown in Figs. 1 and 2. In Fig. 2 the relation between the hourly excretion of phosphate and creatine is obscured on a few occasions by a profuse polyuria which produced high excretion values for all the urinary constituents.

Phosphate. The daily phosphate excretion diminishes with the duration of the fever until the termination either spontaneously or after quinine. Also during each bout of temperature the phosphate excreted diminishes, the maximum fall occurring at the time of the peak temperature. When the fever is well established the phosphate in the urine completely disappears. (No colour was

Fig. 1. The changes in blood and urine during one pyrexial period in three separate subjects. Blood: mg. per 100 ml. Urine: mg. per hour.

Fig. 2. The hourly excretion rate of phosphate, creatine and creatinine in a subject over the duration of an attack of malaria.

formed with Briggs's reagents even with much extra urine.) Immediately after the peak the phosphate rises often to quite high values.

Creatine. The general level of creatine excretion falls somewhat with the full onset of the fever and then returns to normal. After quinine the creatine again

Table I, A, B and C. *To show the variation in the blood and urine phosphate and creatine with change of temperature.*

Date	Time	Temp. °C.	Phos- phate mg.P/ 100 ml.	Crea- tine mg./ 100 ml.	Crea- tine mg./ 100 ml.	Sugar mg./ 100 ml.	Urinary P	Urinary Creatine
E. S.				A.				
20. x. 32	10.50 a.m.	99.0	3.45	6.1	2.6	99	Normal	Normal
	3.40 p.m.	99.4	3.45	7.9	2.8	132	Normal	—
25. x. 32	11.50 a.m.	103.6	3.2	7.2	2.7	93	Low	Normal
	Peak 3.30 p.m.							
	3.50 p.m.	104.4	3.0	4.5	3.8	91	Low	Normal
27. x. 32	11.0 a.m.	105.0	2.1	9.2	4.1	95	Normal	Normal
	12.15 p.m.	105.6	2.5	7.6	2.6	105	Low	High
	2.0 p.m.	105.8	1.8	6.7	3.3	121	Low	High
	Peak 12.30-2.30							
	4.35 p.m.	104.6	2.6	6.7	2.9	99	Normal	High
2. xi. 32	9.30 a.m.	98.6	3.1	5.8	2.4	100	Normal	None
	11.30 a.m.	100.2	3.15	5.1	2.3	100	Normal	None
	3.40 p.m.	105.4	2.6	6.3	2.9	107	Low	Normal
	5.0 p.m.	106.6	1.95	6.3	2.7	105	Low	Normal
	Peak 5.30 p.m.							
	6.30 p.m.	106.0	1.9	5.5	3.0	110	Low	Normal
	8.0 p.m.	104.8	2.25	4.5	3.5	100	Low	Normal
3. xi. 32	11.0 a.m.	98.8	4.05	5.6	2.6	103	Normal	Normal
Quinine given 4.15 p.m. 3. xi. 32								
4. xi. 32	3.40 p.m.	99.2	2.4	5.2	2.1	97	Normal	Normal
5. xi. 32	11.30 a.m.	101.0	2.3	5.6	2.0	114	None	Very low
7. xi. 32	11.20 a.m.	98.4	2.7	5.7	2.0	83	Normal	Normal
							Prev. spec. very low.	Prev. spec. none
8. xi. 32	11.45 a.m.	98.4	3.4	6.0	1.9	135	Low	Low
							Prev. spec. very low	Prev. spec. none
E. G.				B.				
20. iv. 32	11.30 a.m.	98.4	2.9	6.2	2.8	93	Normal	Low
	1.0 p.m.	98.4	2.9	5.3	3.0	75	Lower	Low
	3.0 p.m.	101.0	3.1	6.5	2.8	95	Lower	Low
	5.0 p.m.	106.0	2.0	5.1	3.9	93	None	Low
	Peak 5.0 p.m.							
	7.0 p.m.	103.9	2.0	4.8	4.2	88	None	Normal
	9.0 p.m.	102.0	3.0	4.1	4.1	94	No spec. till	
	11.0 p.m.	100.8	3.5	4.3	4.1	85	6.0 a.m.	
21. iv. 32	10.30 a.m.	98.4	2.8	5.9	3.1	80	High	Normal
26. iv. 32	?	98.4	3.7	9.0	3.0	98	Normal	Normal
G. R.				C.				
14. i. 32	Peak 8.30 a.m.							
	9.40 a.m.	106.5	1.5	—	—	166	None	Normal
	1.0 p.m.	105.2	—	—	—	139	None	Normal
	2.45 p.m.	102.0	4.4	—	—	135	Low	Normal
15. i. 32	3.50 p.m.	98.4	2.8	—	—	124	Normal	Normal
16. i. 32	7.15 a.m.	106.5	1.9	—	—	—	Trace	Normal
	8.15 a.m.	106.8	1.7	—	—	127	Trace	Increasing
Peak	8.15 a.m.							
	9.15 a.m.	106.0	1.9	—	—	93	None	Raised
	10.15 a.m.	105.0	1.5	—	—	93	None	Raised
	11.15 a.m.	104.5	3.0	—	—	110	None	Raised
	12.20 p.m.	102.5	3.8	—	—	104	Low	Raised
	1.15 p.m.	100.8	3.9	—	—	115	Increasing	Low
	2.35 p.m.	98.8	3.9	—	—	99	Increasing	Low

falls. The creatine varies with the temperature. At the time of the rise of temperature there is a rise in the creatine output. A fall starts just after the peak and the creatine output reaches its lowest level at the time when the temperature has just returned to normal. When this coincides with the general low level of excretion no creatine is found in the urine. A similar absence of creatine from the urine occurs after quinine. During the febrile period the minimum values of phosphate and creatine in the urine never coincide, but after quinine they may occur together.

Creatinine. The changes in creatinine excretion are less clear cut. The excretion often runs more or less parallel with that of creatine but at a higher level. There is a tendency for a rise above normal to occur with the rise of temperature but the fall which occurs in the intervals is only slightly below the normal level.

Blood. The changes in the blood are shown in Figs. 1 and 2 and in tabular form in Table I, A, B and C.

Inorganic phosphorus. The blood phosphorus falls to quite low values by the time the peak temperature is reached. The fall starts at the same time as the rise of temperature. There is a corresponding rise in the phosphorus when the peak is passed. The exact time of this rise varies in different cases from the peak to 2 hours after the peak. After quinine low values were obtained.

Creatinine and creatine. The creatine content of the blood rises as the temperature rises. After the peak there is an abrupt fall. The post-critical rise of the blood phosphate does not occur until this fall is complete. Figures are not available for the values in the interval, several days elapsing in every case before another set of observations was made. After quinine all values obtained were somewhat lower than the pre-febrile ones.

Blood sugar. Although the blood sugar was estimated frequently no correlation could be observed between the fever and the blood sugar. The added variable of food which was taken irregularly in relation to the pyrexia and absorbed probably equally irregularly would be sufficient to obscure any underlying relationship unless it were very marked.

These changes are not found solely in malaria since other conditions in which similar bouts of pyrexia alternate with periods of normal temperature also show similar changes. They were shown in a boy suffering from streptococcal septicaemia and to less extent in a child in whom pyrexia was being produced by repeated T.A.B. injections intravenously.

DISCUSSION.

These very striking changes in the phosphate of the urine have been reported as far back as 1860 in malaria by Haxthausen [1860] and many others. A survey of the literature up till 1900 has been made by Garratt [1904]. The findings of all the observers when dealing with malaria were uniform and identical with those reported here. In dealing with non-malarial cases no uniformity of results was obtained, there being no periodic swing to reveal such changes as occur in malaria. The daily output was either slightly increased or diminished at the start and suffered far less variation than did the urea.

No conclusions as to the cause of the changes were advanced by Garratt, but in his discussion he suggests that the phosphorus may have been stored in the muscles and glands of the body. Indeed, no adequate answer could have been given until the recent work on muscle metabolism culminated in the simultaneous discovery of phosphocreatine by Eggleton and Eggleton [1927], and by Fiske and Subbarow [1927].

The precritical period is marked by a rapid rise of temperature and by muscular tremors or rigors which should produce effects similar to muscular work. The effect of work is somewhat different according to its duration and to the degree of fatigue. Short sharp exercise (10 secs.) produces only a fall of blood phosphorus after a few minutes' interval [Gemmill and Ribeiro, 1933], but after exercise of a longer duration (20 mins.) causing exhaustion there is a rise of blood phosphorus followed by a fall. Corresponding but less marked changes occur in the urine, the rise being greater than the fall [Havard and Reay, 1926; Planet and Cardoso, 1933]. There is also a rise in the blood creatine [Kael, 1932; Planet and Cardoso, 1933]. Tetanic stimulation of excised muscle increases the hexosephosphate, but this increase rapidly disappears on cessation of the stimulus [Cori and Cori, 1931; 1933].

Apart from work, adrenaline brings about certain changes. There is an increase of muscle hexosephosphate with no change in the phosphocreatine or adenosinetriphosphate content coupled with a reduction of plasma phosphate and a drop of the urinary phosphate so marked that at times none can be detected [Allan *et al.*, 1924; Cori and Cori, 1931; Bell, 1932].

Another interesting observation is that creatine given by mouth to dogs causes a fall of the blood phosphate and a marked decrease or even complete absence of urinary phosphate [Brown and Imrie, 1931]. Clarkson [1932] has shown that creatine by mouth in man produces a similar diminution of phosphate excretion.

It will be seen that changes detected in the isolated muscle are of such magnitude that in the intact organism considerable changes in the composition of the blood and urine are produced.

In dealing with the febrile cycle of malaria the observation of Borchardt [1928] that the production of experimental fever is considerably modified in the absence of the suprarenal suggests that the secretion of adrenaline occurs in that part of the malarial cycle terminating in the peak. In the same period rigors or severe muscular tremors occur. There is therefore in this phase tetanic muscular contraction superimposed on adrenaline stimulation, which as shown by Cori and Cori [1933] produces a maximum deposition of hexosephosphate. The phosphocreatine broken down by muscular contraction is rapidly reconstituted but when fatigue ensues there is some reduction in its amount, the creatine escaping into the blood and being lost. This is the time at which maximum output of creatine in the urine was found. The phosphate presumably is retained as hexosephosphate. Morais [1932] has shown that in fever produced by dinitro- α -naphthol the phosphocreatine in the muscles of pigeons is reduced from an average of 29.6 mg. to an average of 5.4 mg. per 100 g. muscle.

At the end of this phase, which presumably coincides with the maximum temperature and cessation of rigors, the hexosephosphate due to tetanisation is dissociated, and since the phosphocreatine has been reduced there is ample opportunity for its reconstruction since there is a slight increase in the creatine of the blood and an increase in the available phosphate. This is probably the same type of reaction as occurs when creatine is given by mouth, *i.e.* an increased phosphocreatine formation when the materials are available. The exact time at which the blood phosphate will commence to rise is a resultant of two factors—the rate of breakdown of the hexosephosphate formed by tetanisation and the rate of re-formation of phosphocreatine. At this point there occurs therefore a fall of the blood creatine and a variable response of the blood phosphate. Later on the balance is shifted in favour of a rise in phosphate by the addition of the breakdown products of the extra hexosemonophosphate formed

in response to the adrenaline liberated in the previous phase, this disappearing more slowly according to Cori and Cori [1931]. A reduction in the rate of phosphocreatine formation due to a reduction of the available creatine together with an increase in the phosphocreatine already formed is also a possible factor. It is at this point that the low values for blood and urine creatine were found.

In the early days of the malaria the phosphate of the blood is plentiful and is not completely spent by the time of the peak. All the available creatine can be used to reform phosphocreatine and so minimum values for creatine in the urine are obtained. In the later period the phosphates have decreased in amount and the available phosphate is exhausted by the time the peak is reached. At this point the urine is found to contain little or no phosphate. The amount of phosphate available is now not enough to produce so big a reduction of the creatine in the blood and urine. Why the blood phosphate falls is not clear. It is stated by many authors that the phosphates are excreted in excessive amounts in fever, but in actual fact in these cases there was no increased loss of phosphate compared with the preceding non-febrile period. In many, indeed, less phosphate was lost in the urine. No attempt to determine the loss in the faeces was made and it is therefore possible that the loss occurred there and the results of von Moraczewski [1900] lend some support to this. At the same time there was a variable but definite decrease in the food intake and thus a lessened phosphorus intake.

The increase of nitrogenous output has long been recognised and it has been observed by many [*e.g.* von Moraczewski, 1900] that the ratio P/N falls in fever indicating a retention of phosphorus produced from protein destruction. On the other hand creatinine has been found to be excreted in excess in fever, and since it seems certain that creatine is the precursor to the urinary creatinine there should be no shortage of available creatine. This is borne out by the results obtained here.

The early low creatine values in the urine and the later low phosphate values can thus be accounted for. It is not so easy to account for the simultaneous low values occurring in the days immediately following the administration of quinine and the return of the temperature to normal. These low figures differ from the preceding ones in that they coincide in time and it is only in this period that a specimen of urine was obtained containing neither detectable creatine nor phosphate. These low values did not occur throughout the whole day but occurred on the whole in the period 5 a.m. to noon. The easiest explanation is to suppose that during and at the end of the sleep period there is a regeneration of the phosphocreatine content of the muscles which in all probability were depleted during the attack.

The significance of the absence of creatine and phosphate from the urine is of interest. According to Wigglesworth and Woodrow [1923], Brain *et al.* [1928] and Walker [1932] the kidney has a threshold for phosphate about 2–3 mg. per 100 ml. calculated from the slope of the curve relating urinary and blood phosphate concentration. Walker indeed states that no example in man of reaching this hypothetical threshold has been recorded. It will be seen from Table II that the values of the blood phosphate obtained at times when no phosphate was being excreted agree well with the calculated value but show a fairly wide variation. This variation might be increased by the unavoidable use of whole blood. In the rapid fall which occurs the red cells might lag behind the plasma in regard to the phosphate content and thus misleading high values would occur. If the rate of diffusion were the limiting factor, however, equilibrium would be established almost instantaneously.

Table II. *The level of the blood phosphate in relation to the urine phosphate.*

Phosphate in urine	Blood phosphate (mg./100 ml.)							
	Case	L. S.	S. S.	F. F.	E. S.	J. W.	G. R.	E. G.
None		2.8	3.2, 3.3	2.0	—	—	1.9, 1.7	2.0
Trace		3.2	4.6	—	1.8, 1.9	2.7	2.5	—

In the case of creatine the question is more complex. In the adult no creatine escapes into the urine although it appears in the blood in the same (or even greater) amounts as are found in children actually excreting creatine. Examination of the values obtained here gives no support for a rigid threshold although not enough figures are available to be dogmatic with so rapid a variation of the blood creatine.

Case E. C.	Lowest value of creatine	4.5, 6.5, 4.5.	Urine	+
"	"	5.2	"	0
"	"	6.1	"	Trace
Case E. G.	"	4.2	"	+

It is quite possible that all the chromogenic substance formed after hydrolysis is not creatine, or again the creatine may be in part combined with some larger molecule in its transition from muscle creatine to urinary creatinine, or as suggested by Hunter and Campbell [1918], the greater part of the creatine is in the red cells and only small amounts are present in the plasma. It is probable however that in the rapid variations in the creatine values the excess creatine is in the plasma since it is assumed that the variations are due to the muscles.

SUMMARY.

1. The phosphate, creatine and creatinine in blood and urine have been studied in cases of malaria produced in children.

2. There is a fall of the phosphate in the blood during the rise of the temperature followed by a rise starting about the time the temperature begins to fall. The fall may be so great as to cause the phosphate to disappear from the urine.

3. There is a rise of the creatine in the blood with the temperature followed by a fall as the temperature falls. The creatinine follows the same course only somewhat delayed in time. As with phosphate the fall is great enough to cause the normal creatinuria of children to disappear.

4. When the fever is stopped by giving quinine there is a simultaneous fall of both phosphate and creatine.

5. Evidence for a threshold for renal excretion of phosphate is put forward. The threshold value in these children lies between 1.8 and 3.2 mg. per 100 ml., the lower values being more probable. No evidence of a threshold for creatine could be found.

6. An explanation has been sought on the basis of a rapid breakdown of phosphocreatine and formation of hexosemonophosphate in the muscles by the combined action of adrenaline and muscular action, followed by an equally rapid reversal when the temperature peak is passed.

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specimens of urine required could never have been obtained, and to Miss Dora Ham on whose shoulders the greater part of the analytical work has fallen and who, as well, has shared in the collection of the blood at the various inconvenient hours that have been necessary. I also wish to thank Dr P. Eggleton for helpful criticism of the work.

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