The Effect of Experimental Malaria on Intracellular Concentrations of Sodium and Potassium in Muscle and Liver[1] (34476)

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(Introduced by B. R. Forsyth)
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Overman and his co-workers (1) have described abnormalities in erythrocyte cation metabolism in rhesus monkeys infected with *Plasmodium knowlesi.* These workers observed a progressive increase in intracellular sodium and a concomitant decrease in the intracellular potassium in erythrocytes from malarial animals. More recently, Dunn (2, 3) has confirmed these findings and extended them to other species of hosts and malarial parasites. These more recent investigations have also elucidated the pathophysiology of the rise of intracellular sodium. The erythrocytes from a malarial host were shown to have a diminished active sodium outflux and an increased passive sodium influx. These changes were thought to be secondary to the presence of some circulating toxic material, since the abnormalities of sodium transport occurred in all erythrocytes whether parasitized or nonparasitized.

It, therefore, seemed of interest to investigate other organ systems in search of changes in intracellular cation concentration. If a circulating toxic material existed in malarial infections, it appeared reasonable to assume that intracellular cations in muscle and liver could also be affected. Other authors have speculated as to the existence of increased intracellular sodium in human infections with *Plasmodium falciparum,* but no actual measurements of cellular cations were reported (4, 5). The present study examined the changes of intracellular sodium and potassium in erythrocytes, muscle, and liver during experimental *Plasmodium knowlesi* infections in rhesus monkeys.

Methods. The experimental malarial infections were induced in 2- to 3-kg rhesus monkeys using *Plasmodium knowlesi.* The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. The methods for induction of the infection and for parasite counting have previously been described (2). Red blood cell sodium and potassium were measured in heparinized cells after a triple washing with isosmotic magnesium chloride (2). Erythrocyte, tissue and plasma sodium and potassium levels were determined with an internal standard flame photometer. Plasma and tissue chloride concentration was determined with a Cotlove chloridometer. Identical quantitative techniques were employed for the analysis of muscle and of liver specimens. Muscle specimens were taken from the animals' gastrocnemius muscle, which is characteristically very lean. Liver specimens were taken immediately after exsanguination of the animal. The fresh specimens of liver and muscle were blotted, trimmed of excess fat and connective tissue, and divided into two samples for duplicate analysis. The weight of muscle samples ranged from 2 to 3 g; the weight of liver samples ranged from 8 to 12 g. The duplicate samples were then dried to constant weight over a 24-hr period in a 100° oven and weighed immediately after removal. The dried samples were then ground to a homogeneous powder with a mortar and a Teflon pestle; this powder was diluted with 5% nitric acid. Five milliliters of acid were added for every 100 mg of dried sample. The samples were incubated with the nitric acid for 18 hr in a 38° water

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bath-shaker. The suspension of tissue in acid was then centrifuged at 10,000 rpm for 10 min, and the supernatant fluid was decanted and saved for analysis. The chloride space was used as an estimate of the amount of extracellular fluid trapped in the muscle sample, thereby facilitating the calculation of extracellular sodium and potassium. Although this technique has minor drawbacks, the chloride space has generally been observed to be statistically indistinguishable from the inulin space as an estimate of extracellular fluid in muscle (6). Since more chloride may enter the intracellular space in conditions of disease (7), and since chloride may be present in normal muscle cells in amounts greater than previously recognized (8), our calculations of chloride space may overestimate the extracellular fluid volume and thereby minimize any real changes in intracellular sodium. The hepatic intracellular chloride concentration is unknown (9) and could not be used in the calculation of chloride space; consequently, cation concentrations in liver were expressed per kilogram of wet and dry tissue rather than per liter of intracellular water. The calculations of extracellular water and intracellular sodium and potassium were done according to the method of Graham *et al.* (10). Corrections for Donnan distribution and for the percentage of water in plasma have been applied to all calculations of extracellular anion and cation. The concentration of intracellular chloride in muscle was assumed to be a consequence of the membrane potential as calculated by the Nernst equation; this distribution was assumed to be 24:1, extracellular chloride to intracellular chloride. The rank sum test was employed as a measure of statistical significance in Tables I and II because the variances of the two samples under consideration were not approximately equal (11). Those data in Table I which represent the results of paired experiments on the same monkey, before and after malarial infection, were also analyzed with Student's paired *t* test (11).

Results. Table I shows the results from the examination of separate groups of control and malarial animals. Muscle water content, percentage of parasitemia, plasma sodium and potassium concentrations, erythrocytic sodium and potassium concentrations, and muscle intracellular sodium and potassium concentrations were studied in groups of 9 control and 14 malarial animals. Muscle water content was unchanged from the control percentage (76.6%) in the malarial animals (76.5%). All the animals in the malarial group displayed a significant parasitemia which ranged from 17 to 90% of circulating erythrocytes. No differences were observed between the mean plasma sodium levels in controls and malarial animals; however, monkey No. 911 displayed moderate hyponatremia with a plasma sodium of 128 m*M*. There was a tendency in some of the malarial monkeys to develop mild hypernatremia, probably secondary to poor water intake coincident with the terminal illness. Plasma potassium concentration, however, displayed a significant increase in the malarial animals over the controls; mean control plasma potassium was 3.9 $\pm$ 0.13 m*M* (mean $\pm$ standard error of the mean) as compared to the mean malarial plasma potassium of 5.3 $\pm$ 0.24 m*M*. Control red blood cell sodium was 11.6 $\pm$ 0.92 mmoles per liter of red blood cells, and the red blood cell potassium was 98.6 $\pm$ 0.87 m*M* in the same control animals. In the erythrocytes from the malarial monkeys a significant increase occurred in the sodium concentration, 27.3 $\pm$ 2.7 m*M*, and a decrease was observed in the potassium concentration, 83.1 $\pm$ 3.4 m*M*. The calculated intracellular sodium concentration expressed per liter of intracellular water in muscle exhibited a small but significant increase from a control value of 16.7 $\pm$ .25 m*M* to an experimental value of 18.5 $\pm$ 0.45 m*M*. No significant change was found in the intracellular concentration of potassium in the muscles of the malarial animals. No correlation existed between the increase in erythrocytic sodium and the increase of muscle intracellular sodium. The chloride content of muscle did not differ significantly in the control (71.9 $\pm$ 5.9 mmoles/kg dry weight) from that in malarial animals (64.6 $\pm$ 4.5); however, it is unknown whether the extracellular–intracellular distribution ratio of chloride changes. Paired

TABLE I. Comparison of Muscle Samples from Separate Groups of Control and Malarial Monkeys.[a]

Animal number	Water content (%)	Parasitemia (%)	Plasma (mmoles/liter)		Erythrocyte (mmoles/liter cells)		Muscle (mmoles/liter intracellular H_2O)	
			Na	K	Na	K	Na	K
Controls								
443	77.6		140.0	3.8	13.1	97.4	17.1	149.0
716	75.8		141.0	4.2	11.3	96.2	15.5	163.4
810	75.6		144.0	4.2	11.4	97.7	16.5	152.9
816	77.1		138.5	3.2	18.9	94.3	18.0	168.0
854	76.0		141.5	3.5	9.3	99.4	16.7	162.6
856	75.6		140.0	3.7	9.5	92.6	16.7	142.8
911	77.4		142.5	3.7	8.2	103.3	16.5	157.3
913	77.4		143.5	4.4	11.2	102.0	17.5	163.2
914	76.0		142.0	4.1	11.4	101.0	16.0	166.5
Mean	76.6		141.5	3.9	11.6	98.7	16.7	158.4
SEM	.23		.66	.13	.92	.87	.25	2.8
Malarials								
349	76.3	70	147.0	6.0	43.0	58.3	16.8	158.0
388	75.5	24	139.5	4.9	23.0	92.7	19.9	160.3
606	75.7	90	155.0	5.0	43.9	57.7	19.3	160.3
716	75.9	73	138.0	5.8	16.2	95.6	16.7	174.8
810	78.0	23	144.0	4.2	31.6	75.8	17.8	144.4
816	76.4	90	138.5	5.3	40.9	84.3	20.3	172.3
842	77.7	30	157.0	5.5	24.0	83.2	19.5	150.3
846	76.1	20	156.0	4.6	18.5	88.7	16.9	152.9
854	76.0	17	138.0	5.4	15.4	95.7	20.3	152.8
856	76.4	35	134.0	4.7	22.0	96.1	17.3	149.5
876	75.8	65	145.0	5.4	34.8	74.9	17.1	158.8
911	79.4	88	128.0	7.9	28.1	79.1	21.2	147.0
914	76.3	26	138.5	5.2	19.1	94.1	19.8	157.6
969	76.5	28	152.0	4.8	21.3	87.3	16.5	156.9
Mean	76.5	48.5	143.6	5.3	27.3	83.1	18.5	156.9
SEM	.28	7.7	2.4	.24	2.66	3.4	.45	2.3
Rank sum								
p	NS[b]		NS	.001	.001	.001	.005	NS

[a] All values were obtained by duplicate analysis.

[b] Not significant.

analysis of seven of the animals listed in Table I, that were studied before and after experimental malarial infection, demonstrated a significant increase ($p < .01$) in muscle intracellular sodium and no change in muscle intracellular potassium, results similar to the nonpaired results in Table I. The mean increase in muscle sodium in the paired analysis was 2.5 mmoles per liter of intracellular water or 15.4%.

Plasma sodium and potassium concentration, erythrocytic sodium and potassium concentrations, hepatic content of water, and the sodium and potassium content per kilogram dry and wet weight of liver tissue were studied in three control animals and eight animals with experimental malarial infection. Table II gives these results with the exception of the plasma and erythrocytic values that can be found in Table I (for six of the eight malarial monkeys). The rank sum test was applied as a measure of statistical significance. A significant difference ($p < .01$) between the water content of the control

TABLE II. Comparison of Liver Samples from Separate Groups of Control and Malarial Animals.

Animal number	Water content (%)	Parasitemia (%)	Liver (mmoles per kg dry tissue)		Liver (mmoles per kg wet tissue)	
			Na	K	Na	K
Controls						
457	69.7		84.4	279.6	25.7	84.6
570	67.5		81.7	252.2	26.4	82.0
866	68.0		86.9	251.5	27.8	80.5
Mean	68.4		84.9	261.1	26.7	82.4
SEM	.7		1.1	9.3	.61	1.2
Malarials						
349	74.7	70	154.7	309.5	39.2	78.4
388	72.1	24	122.4	322.9	32.4	85.3
810	76.2	23	182.2	315.8	43.4	75.2
854	73.4	17	143.3	310.0	38.2	82.5
855	72.4	3	144.5	284.8	39.9	78.6
856	73.6	35	147.4	332.4	38.9	87.8
911	76.5	88	173.7	318.8	41.2	75.0
999	75.6	97	156.5	259.9	38.0	63.7
Mean	75.1	44.6	153.0	306.7	38.8	78.3
SEM	.71	12.5	6.6	8.2	1.1	2.6
Rank sum p	.01		.01	.025	.01	NS

(68.4%) and of the malarial monkeys (75.1%) was observed. If the sodium and potassium content per kilogram of dry liver tissue are considered, statistically significant differences between the control and malarial populations were observed for both sodium and potassium. The mean sodium in controls was 84.9 ± 1.1 mmoles per kilogram of dry liver (mean ± standard error of the mean), whereas the value in malaria was 153.0 ± 6.7 ($p < .01$). The potassium in control liver tissue was 261.1 ± 9.3, whereas the value in the liver from malarial animals was 306.7 ± 8.2 ($p < .025$). If the wet weight of the liver is used as a reference value, the difference between control and malarial animals continues to be evident with sodium concentration rising from 26.7 ± 0.61 mmoles per kilogram of wet tissue in the control to 38.8 ± 1.1 in the malarial animals. However, the differences in potassium concentration are not apparent when wet tissue is used as the reference weight. No correlation was found either between liver and muscle sodium or between liver and erythrocyte sodium. Liver chloride content increased from a control value of 18.1 ± 1.5 mmoles per kg wet weight to 23.0 ± 0.9 after malaria, which is equivalent to the addition of 43 ml of extracellular fluid per kilogram of liver.

Discussion. The increase in erythrocytic sodium and decrease of erythrocytic potassium in malarious rhesus monkeys have been discussed extensively by Overman (1) and by Dunn (2, 3) and these erythrocytic cations were measured in this study to examine possible correlations with cation changes in muscle and liver. No quantitative and direct correlations, however, were discovered between the changes of intracellular cations in erythrocytes, muscle, and liver. Although the demonstrated increase of intracellular sodium in muscle of malarial monkeys was significant ($p < .005$), the biological significance of this observation is unclear. The mean increase in intracellular muscle sodium was 1.8 mmoles per liter of intracellular water, or 10.9%, for the analysis of separate groups of controls and malarials, and the increase of muscle sodium was 2.5 mmoles per liter, or

15.4%, for the paired analysis of the same animal before and after experimental malarial infection. This increase in muscle sodium is not of the magnitude of the two- to fourfold increase in red blood cell sodium. In addition, no significant change in intracellular muscle potassium was noted, whereas erythrocytes have consistently displayed a loss of intracellular potassium. This small increase in intracellular sodium is certainly not of sufficient magnitude that it could explain any hyponatremia that may occur either in experimental malarial infections or more commonly in human malarial infections with *Plasmodium falciparum* (4, 5). It should be noted, parenthetically, that an increase in intracellular sodium rarely, if ever, explains hyponatremia. The elevated plasma potassium has been reported by others (5, 12) and probably is a consequence of potassium losses from cells, hemolysis, and impaired renal function.

With regard to our observations in liver, the question arises as to whether the changes in the content of sodium and potassium can be explained by an increased trapping of extracellular fluid in the liver, as manifested by the increase in water content after malarial infection, or whether the increased water content was primarily intracellular. These difficulties would be averted if the chloride space provided an accurate estimate of extracellular space in liver. Harrison's method (13), assuming that all chloride was extracellular, was tried but it occasionally gave negative values for cell sodium. This is not surprising, since others have shown that some chloride in the liver is intracellular (9). The average increase in the water content of the liver tissue from malarial animals was 6.7% of the total specimen weight. If it is assumed that the entire increase in water content is in extracellular fluid, and if we consider the extracellular fluid sodium concentration of 138 m*M* in the eight animals, the observed increase in sodium cannot be solely explained in these terms. A gain of 67 ml of water per kilogram of wet tissue (6.7%) could account for an increase in sodium of 9.2 mmoles per kg wet tissue and the observed average increase in sodium was 12.1 mmoles/kg wet tissue, leaving at least 2.9 mmoles unaccounted for by extracellular fluid. The gain of 5 mmoles of chloride per kilogram of wet liver, which is equivalent to the addition of 43 ml of extracellular fluid, would, at the maximum, increase total sodium content 5.9 mmoles. The fact that tissue water increased 36% more than tissue chloride strongly suggests an increase of intracellular water. In addition, the significant increase of liver potassium per kilogram of dry tissue, in the absence of any change per kilogram of wet tissue, points to the accumulation of intracellular water. Pathologic findings also suggest that the liver's increase of water content in malaria is predominately intracellular (14, 15). These changes in erythrocytes, muscle, and liver are consistent with the hypothesis that a circulating toxic material, detrimental to cellular membranes, is produced during malarial infection. It is perhaps significant that Riley and Maegraith (16) have shown that the serum of monkeys infected with *P. knowlesi* contains a factor which inhibits the respiration and uncouples oxidative phosphorylation of hepatic mitochondria from either mice or monkeys. The changes of sodium concentration in the liver could be secondary either to changes of the metabolism of the hepatic cell or to more direct injury of the liver cell membrane.

It is not surprising that no good correlation was found between the quantitative changes of cellular cation content in the three different organs which were studied. Since there are undoubtedly specific differences in the structure and composition of the membranes of erythrocytes, muscle cells, and liver cells (17), one would expect inherent differences in the response to a disease process. In addition, the effect of the spleen and other reticuloendothelial organs could increase the amount of membrane damage in the erythrocyte (18) whereas the other two tissues would not be subjected to this influence.

Summary. Malaria was induced in rhesus monkeys with *Plasmodium knowlesi.* Intracellular concentrations of sodium and potassium were studied in erythrocytes, muscle,

and liver. The calculated content of sodium increased significantly in these cells, the magnitude of change being greater in erythrocytes than in liver and muscle. The intracellular potassium content decreased in erythrocytes, did not change in muscle, and increased in liver. These data provide additional evidence of a circulating toxic substance in malaria.

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