ize in the heart, and induce the acute and chronic inflammatory reactions associated with the carditis of rheumatic fever.

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Malaria Infection (Plasmodium lophurae): Changes in Free Amino Acids

Abstract. *The course of infection of the avian malaria* Plasmodium lophurae *in the duckling is characterized by striking increase in the intraerythrocytic free amino acid pool. The quality and quantity of this change result from the presence of the parasite and for the most part reflect the free amino acid pool of the growing plasmodium. No significant changes in the free amino acids in plasma were noted during infection.*

The nature of protein synthesis and amino acid metabolism in malaria parasites has been restricted to studies of degradation of hemoglobin or of utilization by the parasites of free amino acids from the plasma. Since plasmodial blood stages must synthesize protein at a rate commensurate with their rapid increase in size and number, it seemed reasonable to conclude, on the basis of previous work, that the erythrocyte amino acid pool was small enough to be insignificant, and that the protein of the erythrocyte was the chief source of amino acids for protein synthesis by the parasites (*1*).

Although erythrocytes parasitized with *Plasmodium knowlesi* and *P. gallinaceum* do accumulate large quantities of amino acids, it is believed that the acids are derived almost exclusively from the proteolysis of hemoglobin. This accumulation of amino acids indicates to some authors that the capacity for hydrolysis of host-cell protein is greater than that for incorporation of the resultant amino acids into parasite proteins (*1*). Two aspects of the problem that have not been clarified are: (i) what are the qualitative changes in free amino acids of infected cells, of infected plasma, and of erythrocyte-free parasites?; (ii) by what mechanism are these changes induced? We now describe the first aspect of this problem.

We worked with the avian malaria *P. lophurae*, which develops synchronous and highly virulent infections in white Pekin ducklings. Blood-induced infections were maintained by the methods of Trager (*2*). Ducks aged 4.5 weeks were starved for 18 hours before blood was withdrawn. Blood samples

Table 1. Free amino acids in malaria-infected (*P. lophurae*) and normal erythrocytes and in free parasites (in micromoles per 100 ml of packed cells).

Acid	Red blood cells						Free parasites*				
	Normal		Infected				Duck (No.)				Mean
	Range	Mean†	Duck (No.)			Mean					
			169‡	171§	172‖		119	120	121	122	
Lysine	9.35–10.5	9.88	21.2	28.2	21.4	23.6	21.4	17.7	13.6	16.4	17.3
Histidine	4.06– 5.15	4.61	9.02	7.07	7.75	7.94	2.54	3.36	3.28	2.18	2.84
Ammonia	20.4 –23.5	21.8	138	77.2	127	114	14.4	9.5	14.8	11.8	12.6
Arginine	3.96– 4.16	4.06	5.6	5.32	4.06	4.99	1.68	2.41	1.75	1.32	1.76
Aspartic	9.9 –13.5	11.35	51.3	72	70.5	64.6	39.3	42.5	33.6	31.9	36.8
Threonine	25.7 –28.7	26.9	28.8	32	43.5	34.7	<2	<2	<2	<2	<2
Serine	42.2 –49.3	46.6	32.3	43.2	53.5	43	<1	<1	<1	<1	<1
Glutamic	14.8 –19.7	17.0	123	193	102	139	152	126	105	112	124
Proline	6.14– 8.26	7.76	12.7	16.9	14.9	14.8	<1	<1	<1	<1	<1
Glycine	67.9 –80	75.8	78.4	97.2	89.3	88.2	18	15.8	11.2	13.5	14.6
Alanine	51.3 –57	53.5	104	127	101	110	9.25	7.6	7.52	5.65	7.50
α-Amino butyric	4.97– 6.72	5.83	8.2	3.65	4.06	5.30	<1	<1	<1	<1	<1
Valine	13.8 –14.55	14.41	20.6	21.3	20	20.6	3.49	2.28	2.28	1.98	2.76
Methionine	3.78– 5.31	4.52	3.84	2.06	3.84	3.25	<1	<1	<1	<1	<1
Isoleucine	7.2 – 8.6	7.6	6.5	7.05	6.25	6.6	1.52	1.55	1.25	0.96	1.32
Leucine	11.8 –12.4	12.1	15.0	16.3	16.9	16.0	3.86	2.34	2.21	1.92	2.58
Tyrosine	12.9 –16.1	14.9	15.5	18.3	14.3	16.0	<1	<1	<1	<1	<1
Phenylalanine	5.0 – 5.83	5.45	8.95	8.23	9.55	8.91	<2	<2	<2	<2	<2
Glutamine		10**				20**	10.8	10.6	7.13	8.15	9.17
		344.07			*Totals*	642.49					241.23

* Value of <1 or <2 indicates that the peak was too small or too broad for acurate measurement of area. † Average of three determinations. ‡ Parasitemia, 51 percent. § Parasitemia, 83 percent. ‖ Parasitemia, 79 percent. ** Not determined accurately because the peak overlapped those of serine and threonine.

Table 2. Free amino acids in normal and malaria-infected (*P. lophurae*) plasmas in ducks (in micromoles per 100 ml of packed cells).

Acid	Normal		Infected			
	Range	Mean*	Duck (No.)			Mean
			169†	171‡	172§	
Lysine	35.5 –38.2	36.6	26.2	32.3	47.4	35.3
Histidine	6.0 – 8.4	7.27	6.1	5.28	8.4	6.59
Ammonia	17.3 –21.7	18.8	28.3	21.5	21.7	23.8
Arginine	13.1 –15.5	14.4	8.65	8.4	13.8	10.2
Aspartic	1.48– 1.56	1.50	1.27	1.61	1.36	1.41
Threonine	28.1 –32.5	29.5	23.5	32.8	40.1	32.1
Serine	49.7 –58	54.5	32.2	52.2	54.8	46.4
Glutamic	8.5 – 9.42	8.8	5.85	10.5	8.76	8.37
Proline	1.55– 1.69	1.62	1.64	1.73	1.97	1.78
Glycine	22.7 –24.7	23.5	24	24.8	31.3	26.7
Alanine	44.6 –55.8	50.1	54.5	60.7	66.6	60.6
α-Amino butyric	6.48– 8.0	7.08	3.42	3.22	5.04	4.89
Valine	20.4 –26.2	22.6	29.4	28.8	32.6	30.3
Methionine	6.84– 8.4	7.86	3.22	3.58	6.73	4.51
Isoleucine	12. –12.4	12.2	9.93	8.1	9.53	9.18
Leucine	18.0 –19.8	18.9	21.9	22	22.8	22.3
Tyrosine	12.7 –16.7	15.0	14.2	18.2	14.2	15.5
Phenylalanine	7.39– 7.9	7.57	9.84	9.25	11.7	10.26

* Average of three determinations. † Parasitemia, 51 percent. ‡ Parasitemia, 83 percent. § Parasitemia, 79 percent.

for amino acid analysis were prepared by standard methods (*3*): 30 ml of blood was withdrawn from the jugular vein into syringes containing 1/10 volume heparin (in physiological saline at 30 mg percent) and centrifuged in the cold (4°C) for 15 minutes at 600*g*; 10 ml of plasma was decanted. The bottom two-thirds of the packed cells were removed by inserting a cannula to the bottom of the tube, thus removing the white cells without washing and allowing the red cells to remain in their own plasma. Infected and uninfected erythrocytes were recentrifuged for 15 minutes at 1300*g* and the plasma was removed with a Pasteur pipette.

Free parasites, untreated with deoxyribonuclease, were prepared by established methods (*4*). Protein was removed by picric acid precipitation. For each 10 ml of plasma, 50 ml of 1-percent picric acid was added. For each 4 ml of cells (erythrocytes and free parasites), 6 ml of distilled water was added; the lysate was shaken and 50 ml of 1-percent picric acid was added. The extracts were centrifuged for 15 minutes at 2000*g*. The protein pellets were discarded and the supernatant was freed of picric acid by running it through a 6- by 2-cm column of Dowex 2. The effluent was lyophilized and brought to 3 ml with distilled water, and the *p*H was brought to 7 to 8 with 1*N* NaOH; it was then treated with 0.5*M* sodium sulfite solution to convert the glutathione to sulfonate. The final volume was 5 ml, with the *p*H brought to 2.2 with citrate buffer. All analyses were carried out on the Beckman-120B amino acid analyzer.

The results for cells appear in Table 1; for plasmas, in Table 2. No striking changes from normal were found in the free amino acid pool of plasma from infected ducklings, in spite of the fact that about 70 percent of the erythrocytes contained parasites. One should note that although the infections were quite severe the animals were not moribund.

The most dramatic changes in the free amino acid pool occur intraerythrocytically in malaria; most of the increase is caused by the free amino acids of the growing parasite. The type and quantity of amino acids found in infected erythrocytes are essentially sums of the contents of the parasite and of the uninfected host cell. Theoretical estimates (*5*) of the total amino acids to be found in infected cells were in close agreement with those observed experimentally. Amino acids that increased most markedly with infection were glutamic and aspartic acids, alanine, and lysine; these same acids were found in highest concentrations in erythrocyte-free *P. lophurae*. Where discrepancies exist between the sum of the contents of the parasite and the normal red blood cell and that in fact observed in infected cells, it appears that the values for the parasite are low—because of the differential leaching of amino acids from the parasites by the five washes in glucose-saline buffer. Such a phenomenon is well known for some amino acids in red cells—for example, alanine, valine, threonine, leucine, and tyrosine (*6*).

There have been few studies of the free amino acids of malaria parasites. The only report (*7*) of qualitative changes in avian malaria indicated an increase in almost every free amino acid in *P. gallinaceum*–infected erythrocytes, but it lacked quantitation; and total amino acids increased similarly. Earlier, Groman (*8*) had shown an approximately threefold increase in α-amino nitrogen during the intraerythrocytic growth of the same parasite; he ascribed these increases to reticulocytosis and hemoglobin hydrolysis by the parasite. According to Groman the production of amino nitrogen was insensitive to anaerobiosis.

Our study confirms and extends these early observations for another malaria, but also shows the qualitative nature of these changes and the location of the increase—in the parasite. Although these data alone do not indicate how the changes in free amino acids arise, work in our laboratory strongly suggests that it is not entirely by way of hemoglobin degradation or uptake of amino acids from the plasma or red cell; that rather they occur by way of CO_2 fixation (*9*). The ultimate fate of these free amino acids in the parasite pool remains unknown.

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Toxicity of Aquatic Herbicides to Daphnia magna

Abstract. *Accurate determination of the acute toxicities of a series of 16 aquatic herbicides to* Daphnia magna *reveals that several of them may present a hazard to food-chain microfauna whether the compounds are environmentally destroyed or not.*

Microcrustaceans such as those of the genus *Daphnia* are of worldwide distribution and represent an extremely important elementary link in the food chain. *Daphnia magna* and *Daphnia pulex* are particularly abundant and form a significant part of the diet of both young and adult fish in ponds and lakes of temperate-zone agricultural areas (*1*).

Toxic effects of a wide variety of insecticides on species of *Daphnia* have been reported (*2, 3*). The low levels of these compounds unintentionally present in bodies of fresh water as a result of drift and run-off from agricultural applications may present a potential threat to organisms of the aquatic food chain through chronic exposure (*4*), but the amounts generally are well below those required for acute intoxication. The median dose for immobilization of *Daphnia magna* by toxaphene is 260 μg/liter (*3*), while the amount of this insecticide typically present in treated lake water, for instance, ranges from 40 to 1 μg/liter (*4, 5*). However, there is an increasing intentional use of herbicides for the direct control of aquatic weeds and algae in lakes, ponds, and waterways, and few published data describe their real or potential effects on aquatic microfauna.

A parthenogenic stock strain was reared for over 50 generations under constant conditions. One hundred female *D. magna* were maintained in each of six 1-gallon (3.8 liters) wide-mouth bottles containing 3.6 liters of deep-well tap water having the following constituents, in milligrams per liter: Ca (as $CaCO_3$), 40; Mg (as $MgCO_3$), 80; Cl, 24; SO_4, 37; B, 0.64; SiO_2, 28; Na, 81; K, 2.2; and Fe 0.0; *p*H was 8.12 and conductivity (μmhos at 25°C), 493. This medium was superior to any of a wide variety of synthetic hard water. Bottles were covered loosely, almost submerged in a glass aquarium at constant temperature, and continuously aerated. Rearing jars were held under constant illumination of approximately 1100 lu/m² from daylight-type fluorescent lamps above; temperature was maintained at 21.1± 0.05°C to produce optimum growth and reproduction. Animals were fed twice daily, by pipette, with 1.5 ml of freshly prepared 3.3 percent suspension (weight to volume) of crushed baker's yeast cake.

In order to obtain test organisms, nylon net was cemented across a flexible circle of plastic tubing so that the resulting disc fitted tightly into each culture jar at a distance of approximately 8 cm above the bottom. Within 15 minutes the normally photopositive first-instar daphnids ascended through the 30-mesh net which excluded almost all larger individuals, and culture water and animals above each net were siphoned into a single reservoir and randomized by agitation. Most of the water was then siphoned back into the original jar through a glass-tipped, 25-mm-diameter tube closed by the 40- to 50-mesh net which excluded all *Daphnia.* Young daphnids were washed several times with boiled deep-well water, held without food for about 2½ hours, and, about 1 hour prior to tests, were passed again through 30-mesh net to remove any that had moulted during the preparation. This convenient technique provided a regular supply of highly uniform animals apparently still similar in characteristics to those collected from nature.

The herbicides were technical standards from the respective manufacturers and were dissolved in nontoxic, reagent-grade acetone when the solubility in water was low. Tests were performed in boiled deep-well tap water to which commercial nonionic surfactant (Tween 20) was added to provide a concentration of 1 part per million (ppm). Twenty-five starved daphnids were transferred by dropper to 100-ml test and control beakers that contained 50 ml of the surfactant solution. Appropriate concentration of toxicant in either acetone or aqueous solution was applied by means of a microsyringe; generally, 50 μl of solution was introduced below the surface, and contents of the beaker were swirled for thorough mixing. After 26 hours, mobility was observed under strong illumination from above and then by illumination from below. Unaffected *Daphnia* swam vigorously toward the light; those that were unable to swim vertically 1 cm or more were considered immobile. Measurement at each concentration was replicated at least three times, data from a log series of dosages were plotted as percentage of immobility versus log concentration on log-probit paper to provide a median immobilization concentration (IC_{50}), and statistical significance was determined by the method of Litchfield and

Table 1. Toxicity, in terms of median immobilization concentrations (IC_{50}), of aquatic herbicides to *Daphnia magna.* Paraquat, diuron, and fenac (Na salt) are not currently registered for aquatic use. Parenthetic values represent 95 percent confidence limits.

Common name	IC_{50} (ppm)	Field use (ppm) (*7*)
Dichlone (Phygon)[a]	0.014 (.011–.017)	0.15
Molinate (Ordram)[b]	.70 (.46–1.05)	3.5
Propanil (Stam, Rogue)[c]	4.8 (3.8–6.6)	7
Sodium arsenite	6.5 (5.7–7.3)	10
Diquat[d]	7.1 (6.3–8.0)	3
Dichlobenil (Casoron)[e]	9.8 (8.8–10.7)	15
Paraquat[f]	11.0 (9.1–12.2)	2
Amitrole[g]	23 (15.3–44.4)	10
Amitrole T[h]	40 (14.3–112.0)	10
Endothall[i]	46 (36–57)	3
Diuron[j]	47 (41.6–53.1)	2
Silvex (K salt)[k]	100	2
Fenac (Na salt)[l]	>100	
Monuron[m]	106	2
MCPA[n]	>100	2
2,4-D[o]	>100	2

[a] 2,3-Dichloro-1,4-naphthoquinone. [b] *S*-Ethyl hexahydro-1*H*-azepine-1-carbothioate. [c] 3′,4′-Dichloropropionanilide. [d] 1,1′-Ethylene-2,2′-dipyridylium dibromide. [e] 2,6-Dichlorobenzonitrile. [f] 1,1′-Dimethyl-4,4′-dipyridylium dichloride. [g] 3-Amino-1,2,4-triazole. [h] Amitrole + ammonium thiocyanate. [i] 3,6-Endoxohexahydrophthalic acid. [j] 3-(3′,4′-Dichlorophenyl)-1,1-dimethylurea. [k] Potassium 2-(2′,4′,5′-trichlorophenoxy)propionate. [l] Sodium 2,3,6-trichlorophenylacetate. [m] 3-(4′-Chlorophenyl)-1,1-dimethylurea. [n] 2-Methyl-4-chlorophenoxyacetic acid. [o] 2,4-Dichlorophenoxyacetic acid.