Coenzyme A Changes in Liver, Spleen and Kidney of Rats with Infections of *Plasmodium berghei.* (22248)

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Previous studies(1) by one of us have shown that survival *in vitro* of the avian malarial parasite *Plasmodium lophurae* was prolonged when concentrates of coenzyme A (Co A) were added to culture media containing the extracellular organisms. Further work (2) has indicated that the parasites were able to accumulate the coenzyme but were unable to synthesize it. In addition, the concentration of Co A in infected chicken livers was approximately 40% lower than in controls, whereas in ducks the drop in concentration of Co A was not so marked. It appeared, therefore, that the depletion of Co A in the liver was correlated with the accumulation of this essential growth factor in the parasites, and in turn was related to the pathology of infection. The present study was undertaken to determine whether a similar situation existed in a mammalian host infected with a malarial parasite. For this purpose observations have been made on the changes in Co A content of livers, spleens, and kidneys of rats during the course of infection with *Plasmodium berghei.*

Materials and methods. The parasite. The Kisonga strain of *P. berghei* was used in this study. This parasite was obtained from Dr. I. H. Vincke and for the past 2 years has been passed biweekly in this laboratory in 40-60 g rats, from the colony of Dr. John B. Nelson, by the intraperitoneal inoculation of whole blood. *The host.* For experimental purposes Sprague-Dawley rats were used. Of 30 animals 25 days old and weighing an average of 48 g, 15 were infected and the remainder kept as controls. Another 30 animals, 116 days old, weighing an average of 204 g, were treated in a similar fashion. To initiate infection each animal was inoculated with 50,000 parasites intravenously. Thereafter, films were taken daily of tail venous blood in order to follow the parasitemia. Infected rats were fed Purina dog checkers and water *ad libitum.* The amount of food and water consumed by each rat was carefully measured every day and averaged for each of the 2 infected groups. The average amount of food and water consumed was then offered to corresponding control animals. Young infected animals usually refused to eat after the first week of infection while older rats appeared to have an unaffected appetite. Restricted dietary intake of control animals was undertaken in order to rule out any possible effects of starvation on tissue Co A content. *The assay.* At 4-day intervals 3 animals from each infected group and 3 from each control group were sacrificed by decapitation. Each rat was allowed to bleed out. An abdominal incision was made and the spleen, liver, and kidney were removed in that order. Immediately upon removal each organ was weighed, placed in a celluloid tube, and then quick-frozen by immersion in a mixture of alcohol and dry-ice. Tissues so frozen were stored in the deep freeze at −25°C until they could be treated for incorporation in the assay medium. Some tissues were stored in this manner for almost 2 months. To prepare for assay each was ground with 4 times its weight by volume of water in a glass homogenizer in an ice water bath. The homogenate was immediately placed in a bath of boiling water for 10 minutes and centrifuged. Co A was determined in the supernatant by microbiological assay, using the methods cited in the work with *P. lophurae*(2).

Results. Essential host-parasite data are described in Table I. In each of the 2 age groups parasitemia followed a course similar to that reported elsewhere(3). In addition changes in the ratio of organ to body weight from the 4th to the 12th days of infection are presented. In young rats the spleen to body weight ratio increased 850% over controls by the 12th day of infection, whereas in old animals the increase was about 600%. At the

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TABLE I. Host-Parasite Data.

Group	No.	Day of infection sacrificed	Age, days	Wt†	Parasites,† 10000 rbc	Ratio liver to body wt*	Ratio spleen to body wt*	Ratio kidney to body wt*
1 X	3	4	29	67	706	.053	.0095	.0062
2 X	3	8	33	94	3410	.067	.028	.0059
3 X	3	12	37	80	6520	.062	.036	.0059
4 X 13	1	16	41	96	<1	.052	.034	.0049
14	1	16		57	12600	.063	.022	.0086
1 Control	3		30	61		.041	.0038	.0053
2 "	3		34	86		.040	.0050	.0049
3 "	3		38	86		.037	.0031	.0046
4 "	2		42	86		.036	.0026	.0041
5 X	3	4	116	208	30	.032	.0043	.0035
6 X	3	8	120	208	550	.039	.012	.0034
7 X	3	12	124	212	720	.037	.017	.0035
8 X	3	16	128	218	1	.034	.013	.0036
9 X	3	20	132	223	<1	.035	.0090	.0037
5 Control	3		117	200		.028	.0027	.0037
6 "	3		121	203		.026	.0025	.0035
7 "	3		125	212		.029	.0021	.0035
8 "	3		129	211		.026	.0023	.0037
9 "	3		133	223		.031	.0024	.0036

* Avg. † Avg for surviving animals.

same time, the liver to body weight ratio in young animals was 63% greater, and one half that value in old animals. The kidney to body weight ratio showed an increase of 25% in the young animals and no change in old rats.

Co A content of liver, spleen, and kidney at various stages during the course of infection is described in Table II. In the livers of young rats, a drop in Co A concentration was evident by the 8th day of infection. The total Co A present remained about the same even though liver to body weight ratios were 50% greater than in controls. The average percentages dry weight for the livers of 4 infected animals and 4 control animals sacrificed on the 8th day of infection were respectively 25.6% and 28.7% indicating that the drop in concentration of Co A was not simply dilution due to higher water content. In the older animals neither the decrease in coenzyme concentration nor the increase in liver size was so marked. In the spleen, on the other hand, the concentration of Co A remained constant through the course of infection, while the total amount present was proportional to the size of the organ. The kidneys of young rats showed a slight drop in Co A concentration with no change in the total, while in the older animals no changes were apparent in either organ size or Co A content.

It should be mentioned here that no differences were noted in concentrations of free pantothenate found in the livers or spleens of experimental and control animals. In both young and old groups the range for liver was 4-10 μg per g tissue. In spleen the range for young animals was 2-3 μg per g and in the older groups 1-3 μg per g. In the kidneys, both infected and uninfected old rats showed a range of 19-26 μg/g, whereas infected young rats had concentrations of 6-11 μg/g as contrasted to 12-28 μg/g in uninfected young animals.

Discussion. Changes observed in liver Co A of rats infected with *P. berghei* were similar to those described for ducks with infections of *P. lophurae.* In both cases there was a marked drop in the concentration of Co A, but the concomitant enlargement of the liver resulted in a total Co A content approximating that of controls. However, in chickens infected with *P. lophurae,* both the concentration and total amount of Co A in the liver were reduced. It is of interest to note the results observed with rats 13 and 14 in group 4 X (Table II). Rat 14, sacrificed at the terminal stages of infection, showed a decrease in both concentration and total Co A,

TABLE II. Average Units of Coenzyme A

Group	Category*	Liver		Spleen		Kidney	
		Per g†	Per organ	Per g†	Per organ	Per g†	Per organ
1 X	Infected—Young	163	580	20	13	55	24
2 X		98	594	21	54	56	30
3 X		103	512	21	60	51	24
4 X 13		157	790	24	78	67	32
14		81	301	19	24	58	29
1 C	Restricted diet controls	192	486	20	5	67	22
2 C	—Young	174	596	18	8	68	29
3 C		162	522	18	5	72	28
4 C		172	509	19	5	53	19
5 X	Infected—Old	112	737	19	18	59	44
6 X		110	851	17	54	36	27
7 X		110	848	23	69	47	34
8 X		109	813	23	68	54	42
9 X		93	728	20	39	74	60
5 C	Restricted diet controls	129	713	15	8	54	40
6 C	—Old	135	704	14	7	49	35
7 C		151	913	21	9	50	37
8 C		126	701	17	9	60	46
9 C		112	760	18	9	47	38

* For more complete data on course of infection see Table I. † Fresh wt.

despite its much enlarged liver. Rat 13, killed at the same time, was well on its way to recovery. Its liver had a normal concentration of Co A, but was still considerably enlarged, so that the total Co A was above that seen in controls.

The short period of reduced food intake which accompanied the infection in young animals, and which was artificially produced in the corresponding uninfected group, evidently had little, if any, effect on the Co A concentration of the liver. Average Co A concentration in the 6 livers of rat groups sacrificed on the 8th and 12th day, at the height of infection, showed a large decrease in infected animals and values for controls intermediate to those reported in the literature. Livers of young infected rats contained 100 units of Co A per g and mature infected rats 110 units. Of the controls the young animals showed 168 units, whereas the older ones had 143 units per g liver. The disparity among values cited in the literature(4-6) and with these might be due to the different strains of rats used.

Kidneys of rats infected with *P. berghei* exhibited no appreciable changes in either the concentration or total Co A present. However, there was a marked difference in the concentration of free pantothenate in the kidneys of young infected and control animals. This may have resulted from increased reabsorption of free pantothenate in the infected group because of the heavy demands of the parasite on the host for Co A.

In contrast to the liver, Co A concentration in spleen remained constant whereas the total amount present was proportional to increase in size of the organ. This suggests that the cells involved in hyperplasia of the spleen, in response to the infection, have the same Co A content as those originally present, and is in accordance with the finding that spleens of chickens with infections of *Plasmodium gallinaceum* showed no changes in glucose metabolism(7).

In view of both present and past work, it seems that the ability to produce Co A may rank as one of those factors of innate immunity which determine the ultimate death or survival of the host. When the demands of the parasite are so great that the host cannot supply its own tissues with sufficient Co A, the animal succumbs, whereas the animal will survive if it can maintain the coenzyme supply until the forces of acquired immunity control the infection. In addition the decreased concentration of Co A in the livers of infected animals might be responsible for the decreased ability of that organ to convert

glucose to glycogen(8). In any case, the observed changes are in harmony with the hypothesis that in mammalian as well as in avian malaria, disturbance in Co A metabolism is significant in the pathogenic effects of the infection.

Summary. Co A concentration and total content in liver, spleen, and kidney of normal rats and rats with infections of *Plasmodium berghei* have been described. During the course of infection the concentration of Co A in the liver dropped but the total amount present was unchanged. In the spleen the concentration remained static, whereas the total was proportional to the increased size of the organ. The kidney showed no appreciable changes in Co A, but a decreased concentration of free pantothenate was apparent in young infected animals.

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Inhibition of Mumps and Influenza B Virus Multiplication by Synthetic Poly-D-Lysine.* (22249)

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It has been previously reported that the synthetic basic polypeptide, poly-L-lysine, protects the chick embryo for a period following inoculation with a number of animal viruses such as mumps virus(1), infectious bronchitis, Newcastle disease viruses(2), and influenza B virus(3). A comparative study using a synthetic basic polyelectrolyte, as polyvinylamine, showed that this polymer was not as effective a virus inhibitor as poly-L-lysine and was almost 5 times more toxic to the chick embryo(3). Proteolytic enzymes (4) in the chick embryo have been demonstrated to destroy poly-L-lysine injected into the allantoic fluid(5). Since this enzymatic destruction of the L-polypeptide might be a factor that limits its protective action against viruses and as D-polypeptides are usually less susceptible to enzymatic hydrolysis, we have studied the effectiveness of poly-D-lysine against mumps and influenza B viruses in the chick embryo.

Materials and methods. The mumps and influenza B Lee strain viruses† were cultivated in chick embryos according to a previously described procedure(1,3). The virus cultures and the procedures for inoculation, harvesting and estimation of the extent of virus multiplication by hemagglutination titrations, were similar to those used by Green and Stahmann(1,3). In all experiments fertile White Leghorn eggs were employed. Poly-D- and poly-L-lysine hydrochlorides of molecular weights of about 3,900 and 3,600 respectively (determined by end group analysis) were prepared by methods published elsewhere(6-8). The maximum non-lethal dose of poly-D-lysine was approximately 0.1 mg and that of poly-L-lysine was 1.0 mg per chick embryo.

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