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Changes in Ascorbic Acid Metabolism of the Rat During Infection with *Trypanosoma hippicum.**

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Investigation of the biochemical changes occurring in the host during trypanosomiasis has been directed primarily toward the observation of changes in carbohydrate metabolism.[1] There have been some studies, however, which indicate that the ascorbic acid metabolism of the host is affected during infection. Scheff and Csillag[2] showed that the ascorbic acid content of the liver and blood of guinea pigs is lowered during infection with *Trypanosoma brucei.* Ascorbic acid administration was reported by Perla[3] to increase the resistance of guinea pigs to infection with *T. brucei,* but not to alter the course of the infection in mice. These findings sup-

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1 VonBrand, T., *Quart. Rev. Biol.,* 1938, **13**, 41.

2 Scheff, G., and Csillag, Z., *Arch. f. exp. Path. Pharmakol.,* 1938, **183**, 467.

3 Perla, D., *Am. J. Hyg.,* 1937, **26**, 374.

ported the report made by Borghi[4] that scorbutic guinea pigs showed an increased susceptibility to infection with *T. brucei*.

The present study was undertaken to investigate alterations in the content of the tissue ascorbic acid of rats infected with *T. hippicum*. The distribution of oxidized and reduced ascorbic acid was studied in the hope that analysis for both forms would yield some information leading to a better understanding of the biochemical changes observed during trypanosomiasis.

Methods and Materials. Male Sprague-Dawley rats weighing 200-300 g were kept on a uniform diet[5] 3 weeks previous to use in order to attain a constant level of ascorbic acid in the tissues. The animals were divided into two groups. The first, or control, group was sacrificed at the end of the 3 weeks feeding period, while the second group was infected after this feeding period and then maintained on the same diet during the course of infection. Both normal and infected animals were fasted 24 hours before their tissues were taken for analysis. The strain of *T. hippicum* used† was maintained by passage through rats, which were infected by intraperitoneal injection of the trypanosomes suspended in Ringer-Locke solution at pH 7.4. About 36 hours after infection the level of the parasitemia was determined by hemocytometer. From this information is was possible to predict the time of death to within 6 hours of its occurrence which was, on the average, 60 hours after infection. The infected rats were sacrificed after pre-mortal symptoms appeared, but before the terminal convulsions occurred.

Samples of liver, spleen, adrenal, skeletal muscle and plasma were taken for analysis. In the assay of the adrenals, one gland was used for the determination of oxidized ascorbic acid and the other for total ascorbic acid. Plasma, free of trypanosomes, was obtained by differential centrifugation of heparinized blood collected after decapitation of the animals. The tissues were homogenized[6] in a 5% trichloroacetic acid for total ascorbic acid, or in 6% metaphosphoric acid containing 0.5% thiourea for dehydroascorbic acid analyses. Samples of plasma were added to similar acid solutions. Dehydroascorbic acid was measured in the acid extracts by the method of Roe and Oesterling[7] modified by Herrmann and DuBois,[8] and total ascorbic acid was measured by the method of Bessey, Lowry, and Brock.[9] The spectrophotometric measurements were carried out on a Coleman spectrophotometer at 520 mμ wave length. Values for reduced ascorbic acid were calculated by subtracting the values for dehydroascorbic acid from those of the total.

All values for tissue ascorbic acid were expressed as mg of ascorbic acid per g of dry tissue. There was no significant difference between the water content of the tissues of the infected rats and that of the control rats. The results of the plasma analyses were expressed as mg of ascorbic acid per g of plasma. Means were calculated for both the control and infected animals, and the significance of the results was evaluated by calculating the "t" values between the means of the experimental and control data, in which

$$t = \frac{m_1^2 - m_2^2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}.$$ [10]

Results. The figures in part 1 of the table show that a large decrease was observed in the total ascorbic acid content of the spleen. The greatest proportionate decrease took place in the reduced fraction, while the dehydroascorbic acid showed a slight, but not very significant, decrease in the infected animals. In the course of these experiments it was

4 Borghi, B., *Atti accad. Lined,* 1933, **17**, 665.

5 Hasch, Z., and Hajdu, I., *Pfliigers Arch.,* 1939, **241**, 507.

† The strain of *T. hippicum* used in these experiments was kindly supplied by Dr. M. H. Soule, University of Michigan.

6 Potter, V. E., and Elvehjem, C. A., *J. Biol. Chem.,* 1936, **114,** 495.

7 Roe, J. H., and Oesterling, M. J., *J. Biol. Chem.,* 1944, **152,** 511.

8 Herrmann, E,., and DuBois, K., unpublished.

9 Bessey, O., Lowry, O., and Brock, M. J., *J. Biol. Chem.,* 1947, **168**, 197.

10 Burn, J. H., Biological Standardization, Oxford University Press, London, 1937, 29.

observed that the ratio of the wet weight of the spleen to the total body weight increased from 1.94×10^{3} in the controls to 5.46×10^{-3} in the infected rats, an increase of 281% in the spleen to body weight ratio. When the total amount of ascorbic acid was calculated from the weight of the total spleen and the concentration of ascorbic acid in mg per g of *wet* tissue, if was found to increase only 175%. This indicated that the decrease observed in the spleen was an actual diminution in the ascorbic acid content, and was not due to a dilution of the ascorbic acid normally present as the result of splenic hypertrophy. The results of the ascorbic acid analyses on the adrenals are presented in part II of the table. The total ascorbic acid of the adrenals of the infected animals decreased significantly. The primary alteration occurred in the reduced fraction, while the dehydroascorbic acid showed no change from normal. Since the ascorbic acid of the adrenal is concentrated in the cortex, the change represented by these results would be more pronounced if the determination were based on the cortex alone, rather than upon the entire adrenal.

The changes which took place in the liver ascorbic acid are shown in part III of the table. The total ascorbic acid decreased during infection. As in the spleen and adrenal, the greatest proportionate decrease occurred in the reduced form. The dehydroascorbic acid of the liver of the infected animals was also less than that observed in the controls, but this decrease was not statistically significant.
The ascorbic acid concentration of the muscle was not greatly affected by infection with *T. hippicum*. It can be seen from part IV of the table that, although the muscle dehydroascorbic acid increased slightly, the significance of the increase ($t = 2.9$) is open to question.

The figures in part V of the table show that the ascorbic acid of the plasma was elevated above normal. Unlike the tissues discussed previously, both fractions were increased to the same extent. The dehydroascorbic and reduced ascorbic acid each increased 100% in the plasma of the infected rats.

Discussion. The lowered concentration of reduced ascorbic acid in the spleen and adrenal, without an equivalent decrease in oxidized ascorbic acid, indicates that there was an interference in the oxidation-reduction processes of the host during infection, which may have been responsible for the changes observed in the distribution of ascorbic acid in the infected animals. These findings are comparable to those of Scheff and Csillag[2] who found that the reduced glutathione decreased while the total glutathione did not change in the blood and liver of guinea pigs infected with *T. brucei.* To a lesser extent, a similar decrease took place in the ratio of reduced to oxidized ascorbic acid in the muscle, because of the increase observed in dehydroascorbic acid. In the liver and plasma, the normal ratio was not measurably altered.

The decrease in ascorbic acid in all tissues studied, except muscle, accompanied by an increase in plasma ascorbic acid, suggested that the metabolite may have diffused from the tissues to the blood. This explanation does not seem wholly adequate, since a greater decrease took place in the reduced fraction in the depleted tissues, while both forms of ascorbic acid increased in the plasma.

Our knowledge of the metabolic requirements of trypanosomes is limited, but it is of interest to note that Groat and Mora[11] found that their strain of *T. cruzi* required reduced ascorbic acid for cultivation *in vitro.* Further investigation of the changes produced in the oxidation-reduction systems of the host by *T. hippicum* may reveal the significance of the alterations in the distribution of ascorbic acid observed and their relation to the symptomology of the infection.

Summary. Dehydroascorbic and total ascorbic acid were measured in the liver, spleen, adrenals, muscle, and plasma of rats infected with *T. hippicum* and the reduced ascorbic acid content of these tissues was calculated.

11 Groat, H., and Mora, C., *Anales soc. biol. Bogota,* 1947, **2**, 188.

TABLE I, The Effect of *T. hippicum* Infection on Ascorbic Acid Content of Rat Tissues. Ascorbic acid content is expressed as mg per g of dry tissue. In the spleen, adrenal, liver and muscle, 7 control animals and 7 infected animals were used. In the analyses made upon the plasma, 2 separate groups of 8 animals each were used.

	Animal No.	Total mg/g		Dehydro. mg/g		Reduced mg/g (Total-dehydro.)	
		Control	Infected	Control	Infected	Control	Infected
Spleen I	1	3.22	1.75	0.59	0.34	2.63	1.41
	2	3.04	2.14	0.46	0.27	2.58	1.87
	3	2.83	1.64	0.20	0.34	2.63	1.30
	4	3.14	1.72	0.35	0.19	2.79	1.53
	5	3.00	1.72	0.30	0.23	2.70	1.49
	6	2.79	1.96	0.36	0.26	2.43	1.70
	7	2.92	2.07	0.33	0.26	2.59	1.81
	mean	2.99	1.86	0.37	0.27	2.62	1.59
	ϵ	0.06	0.07	0.05	0.02	0.06	0.08
	t*	11.9		2.0		10.3	
Adrenal II	1	12.75	11.41	0.99	1.03	11.76	10.38
	2	14.96	8.77	1.24	0.52	13.72	8.25
	3	—	11.65	1.26	1.39	—	10.25
	4	12.67	9.02	0.83	0.98	11.84	8.04
	5	13.50	8.31	1.02	0.82	12.48	7.49
	6	10.98	10.25	0.91	1.17	10.07	9.08
	7	13.74	8.77	0.71	0.74	13.03	8.03
	mean	13.10	9.70	0.99	0.95	12.15	8.79
	ϵ	0.54	0.51	0.08	0.11	0.52	0.43
	t	4.6		0.31		5.0	
Liver III	1	1.22	1.01	0.29	0.32	0.93	0.79
	2	1.38	0.66	0.23	0.09	1.12	0.57
	3	1.28	1.03	0.35	0.23	0.93	0.80
	4	1.10	0.84	0.12	0.08	0.98	0.76
	5	1.59	0.80	0.15	0.08	1.43	0.72
	6	1.08	0.78	0.17	0.11	0.91	0.67
	7	1.17	0.67	0.13	0.13	1.04	0.54
	mean	1.26	0.83	0.21	0.15	1.05	0.69
	ϵ	0.07	0.06	0.03	0.03	0.07	0.04
	t	4.90		1.4		4.5	
Muscle IV	1	0.18	0.23	0.06	0.16	0.12	0.07
	2	0.17	0.33	0.08	0.10	0.09	0.23
	3	0.20	0.20	0.07	0.14	0.12	0.07
	4	0.25	0.20	0.07	0.06	0.18	0.14
	5	0.20	0.24	0.07	0.06	0.12	0.17
	6	0.20	0.20	0.07	0.09	0.12	0.11
	7	0.18	0.20	0.07	0.09	0.11	0.11
	mean	0.20	0.23	0.07	0.10	0.12	0.13
	ϵ	0.01	0.02	0.003	0.01	0.01	0.02
	t	1.8		2.9		0.44	
Plasma V†	1	0.015	0.041	0.010	0.017	0.005	0.024
	2	0.025	0.042	0.016	0.032	0.009	0.010
	3	0.017	0.050	0.012	—	0.005	—
	4	0.023	0.046	0.009	0.028	0.014	0.018
	5	0.019	0.042	0.014	0.033	0.005	0.009
	6	0.026	0.050	0.015	0.039	0.011	0.011
	7	0.016	0.051	0.008	0.021	0.008	0.030
	8	0.020	0.032	0.017	0.020	0.003	0.012
	mean	0.020	0.044	0.013	0.027	0.008	0.016
	ϵ	0.002	0.003	0.001	0.003	0.001	0.003
	t	9.6		4.4		2.7	

*$t = \dfrac{m_1 - m_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$

† mg ascorbic acid/g of plasma.

The liver, spleen and adrenals showed significant decreases in reduced ascorbic acid content. The dehydroascorbic acid content of the muscle was increased slightly and both dehydroascorbic and reduced ascorbic acid of the plasma were increased two-fold.

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