3. O. Rampersad and I. G. Wool, *Science* **149**, 1102 (1965); I. G. Wool, O. Rampersad, A. N. Moyer, *Amer. J. Med.* **40**, 716 (1966).
4. D. M. Kipnis and M. W. Noall, *Biochim. Biophys. Acta* **28**, 226 (1958).
5. H. E. Morgan, M. J. Henderson, D. M. Regen, C. R. Park, *J. Biol. Chem.* **236**, 253 (1961).
6. R. Scharff and I. G. Wool, *Biochem. J.* **97**, 257 (1965).
7. H. A. Krebs and K. Henseleit, *Hoppe-Seylers Z. Physiol. Chem.* **210**, 33 (1932).
8. G. von Ehrenstein and F. Lipmann, *Proc. Nat. Acad. Sci. U.S.* **47**, 941 (1961).
9. I. G. Wool and P. Cavicchi, *ibid.*, in press.
10. J. L. Kostyo, *Endocrinology* **75**, 113 (1964).
11. Supported by Life Insurance Medical Research Fund, the John A. Hartford Foundation, NIH (AM-04842), and a PHS research career development award (to I.G.W.). We thank Dr. O. Rampersad, P. Cavicchi, and A. N. Moyer for advice and assistance.

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Rheumatic-like Cardiac Lesions in Mice

Abstract. *A single intraperitoneal injection of a sterile extract of sonically disrupted group A streptococcal cells induced an inflammatory process in the hearts of mice. The cardiac lesions, in terms of their distribution and histological features, are similar to the cardiac lesions of rheumatic fever.*

The properties of a toxic moiety found in extracts of sonically disrupted group A streptococcal cells have been described (*1*). The inflammatory reaction of rabbits that had been given a single intradermal injection of this toxic moiety was characterized by remissions and exacerbations observed over a 90-day period. The toxic material appeared to be cell wall fragments of a limited range of particle sizes, made up of C carbohydrate and mucopeptide (*2*). These observations served as a basis for the concept that fragments of group A streptococcal cell walls can act as durable toxic materials and can induce the acute and chronic inflammatory reactions associated with nonsuppurative sequelae of group A streptococcal infections, including rheumatic fever. This concept has been tested in experiments in which extracts of sonically disrupted group A streptococcal cells (type 3, strain D58) were injected intraperitoneally into mice.

Each of 54 Swiss Webster mice weighing from 20 to 22 g was injected intraperitoneally with 4 ml of sterile crude extract of sonically disrupted group A streptococcal cells suspended in 0.038*M* phosphate buffer *p*H 7.6. The extract contained 890 μg/ml of rhamnose (*3*). One group of controls consisted of 54 mice, each of which was injected with 4 ml of crude extract of sonically disrupted group D streptococcal cells suspended in the same buffer. This extract contained 899 μg/ml of rhamnose. A second group of controls consisted of 48 mice, each of which was injected with 4 ml of the buffer in which the cell extracts were suspended. Twenty-nine mice that received the group D extract died within 96 hours after injection. The mice that received buffer and the mice that received group A streptococcal extract were divided into groups and sacrificed at 1, 2, and 3 days, and at weekly intervals for 8 weeks after injection. Groups of mice that survived injection of group D streptococcal extract were sacrificed at 1, 2, and 3 days and at weekly intervals for 6 weeks after injections. Blocks of tissues were fixed in 10 percent buffered formalin, and sections were stained with hematoxylin and eosin.

The extracts of group A and D streptococcal cells stimulated a generalized acute fibrinopurulent peritonitis which evolved into a chronic focal granulomatous process and continued throughout the 8-week period of observation in animals receiving extract of group A cells. The chronic peritonitis was not observed in animals injected with the extract of group D cells and sacrificed 4 to 6 weeks later. Both extracts caused focal granulomas of the liver. Granulomas of the group D material were not observed after 4 weeks, although they continued to be present for 8 weeks in animals receiving extracts of group A cells. The spleens of both groups of animals showed an acute inflammatory reaction in animals sacrificed during the first 3 days of the experiment. No chronic focal lesions of the spleen were observed. In kidneys from animals sacrificed 2 and 3 weeks after injection with extracts of group A cells, a moderate degree of thickening of the stroma of the glomeruli was associated with an increase in glomerular cells and a decrease in the blood in the glomerular capillaries. Kidneys from the other animals showed no lesions.

With the exception of a single microscopic focus of interstitial inflammation seen in the myocardium of one mouse sacrificed for study 48 hours after injection, no lesions were seen in the hearts or other organs of the animals injected with buffer. Hearts of animals that were injected with extracts of group D cells and sacrificed for study 1, 2, 3, and 7 days later showed scattered microscopic foci of myofiber degeneration and necrosis associated with accumulation of neutrophils and mononuclear cells. This acute reaction was limited to the myocardium and did not evolve into a chronic process. Hearts of animals of this group sacrificed for study 2, 3, 4, 5, and 6 weeks after injection showed no lesions.

No lesions were observed in the hearts of mice sacrificed 1 day after inoculation with extracts of group A streptococcal cells. Sections of the hearts of animals injected with extracts of group A streptococcal cells and sacrificed 2 days later revealed foci of myofiber degeneration and necrosis associated with accumulations of mononuclear cells. Hearts collected for study 3 days to 8 weeks after injection of extracts of group A streptococcal cells revealed an inflammatory process that involved the pericardium, myocardium, and endocardium of the left atrium and left ventricle, the coronary arteries, the mitral and aortic valves, and the proximal portion of the aorta. Focal inflammatory lesions of the pericardium consisted of an accumulation of neutrophils and mononuclear cells in edematous subepicardial tissue, and adjacent myocardium and collections of similar cells on the surface of the epicardium. Lesions of the myocardium observed in animals sacrificed 3 days to 2 weeks after injection were characterized by foci of necrosis surrounded by a variety of cells, including mononuclear and giant cells with irregular basophilic cytoplasm, indistinct cell borders, and nuclei of the type observed in Anitschkow cells (*4*) (Fig. 1). The granulomatous lesions observed in the hearts of animals sacrificed later in the experiment were similar to those described; the only differences were that the areas of necrosis were less prominent and there was an increase in the fibroblasts in and around the lesions. Some of the focal granulomatous lesions of the myocardium were adjacent to or surrounded a coronary artery (Fig. 2); others were not clearly related to an artery (Fig. 1). Changes in the cusps and roots of the mitral and aortic valve were noted in animals sacrificed 3 days to 8 weeks after injection of extracts of group A streptococcal cells (Figs. 3 and 4). There was edema of the stroma of the valves associated with accumula-

tions of neutrophils and mononuclear cells including Anitschkow cells (Fig. 3). In addition to diffuse valvulitis, there were focal nodular lesions (Fig. 4). In these focal lesions there was necrosis involving both the fibrosa and spongiosa of the valves with dense accumulations of inflammatory cells. The roots of the mitral and aortic valves and adjacent myocardium showed areas of necrosis and accumulations of a variety of cells similar to those seen in the granulomatous lesions of the myocardium described above. The endocardium of the left ventricle and left atrium adjacent to the mitral valves showed focal lesions characterized by hyperplasia of endocardial cells and accumulations of inflammatory cells. Lesions of the proximal portion of the aorta were characterized by a proliferative and exudative reaction involving the endothelium, media, and adventitia. Inflammatory changes of the coronary arteries of varying severity occurred in mice sacrificed 3 days to 8 weeks after injection, and were characterized by hyperplasia of the endothelium, edema of the media, and accumulations of mononuclear cells in the media and adventitia (Fig. 2). Thrombi were not observed in any of the arteries.

The inflammatory process was extensive in the hearts of all animals injected with extracts of group A streptococcal cells and sacrificed for study 3 days to 4 weeks after inoculation. The process appeared to be subsiding in animals sacrificed 5 to 8 weeks after inoculation. However, isolated granulomatous lesions of the myocardium and scarring and chronic inflammation of the cusps and roots of the aortic and mitral valves were found in hearts collected during the last 4 weeks of the experiment.

Comparison of the experimental carditis induced in mice by injecting extracts of sonically disrupted group A streptococcal cells with the carditis of rheumatic fever reveals a number of similar features. The distribution of the lesions with involvement of the pericardium, myocardium, endocardium, valves, coronary arteries, and proximal portion of the aorta is similar in the experimental model and in rheumatic carditis (*5*). More extensive involvement of the left side of the heart is another characteristic shared by rheumatic fever and the experimental disease. The histologic features of the pericarditis, arteritis, valvulitis, and endocarditis observed in the experimental animals are similar to those seen in rheumatic fever (*5*).

The lesion considered pathognomonic for rheumatic fever is the Aschoff body (*6*). The focal granulomatous lesions of the myocardium observed in the mice inoculated with extracts of sonically disrupted group A streptococcal cells have features in common with Aschoff bodies which include their location, the presence of foci of necrosis, and the accumulation of a variety of cells, some with the characteristics of Anitschkow cells (*4*). Final judgment as to whether these lesions are true Aschoff bodies (*6*) can not be made at this time.

The observations recorded above do not make known the nature of the toxic moiety responsible for the carditis observed in mice injected with crude extracts of sonically disrupted group A streptococci. The type of histological reaction produced, and the rather prolonged course of the process following a single injection, suggest that this moiety may be cell-wall fragments which have been shown to cause the prolonged inflammatory reaction observed in the skin of rabbits, following the direct injection of the crude extract (*1*). The idea that the toxic moiety consists of fragments of cell walls is supported by an extension of these studies being carried out with Schwab (*7*). These studies have shown that purified group A streptococcal cell-wall fragments, when given as a single intraperitoneal injection, induce a similar carditis in mice.

The findings reported are in keeping with the concept that fragments of group A streptococcal cell walls can act as durable toxic material, local-

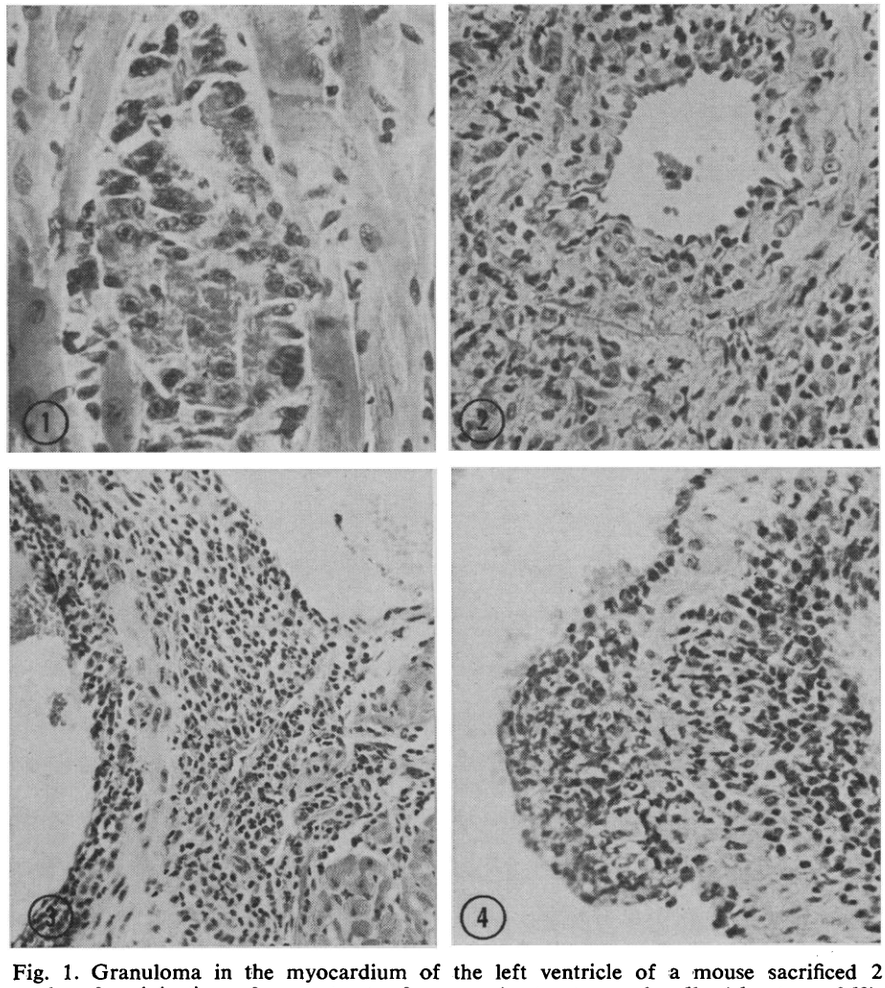

Fig. 1. Granuloma in the myocardium of the left ventricle of a mouse sacrificed 2 weeks after injection of an extract of group A streptococcal cells (about × 360). Fig. 2. Arteritis in a heart collected for study 8 weeks after injection of an extract of group A streptococcal cells. This section also shows a portion of granulomatous lesion that surrounded the artery (about × 360). Fig. 3. Inflammation of the cusp and root of the mitral valve from a mouse sacrificed 1 week after injection of an extract of group A streptococcal cells (about × 235). Fig 4. Nodular lesion of the mitral valve cusp from a mouse sacrificed 3 days after injection of an extract of group A streptococcal cells (about × 360).

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

WILLIAM J. CROMARTIE
JOHN G. CRADDOCK
Departments of Bacteriology and Medicine, University of North Carolina School of Medicine, Chapel Hill

References and Notes

1. J. H. Schwab and W. J. Cromartie, *J. Bacteriol.* **74**, 673 (1957); W. J. Cromartie, J. H. Schwab, J. G. Craddock, *Amer. J. Pathol.* **37**, 79 (1960); W. J. Cromartie, in *The Streptococcus Rheumatic Fever and Glomerulonephritis*, J. W. Uhr, Ed. (Williams and Wilkins Company, Baltimore, 1964), p. 187.
2. B. S. Roberson, J. H. Schwab, W. J. Cromartie, *J. Exp. Med.* **112**, 751 (1960); E. M. Abdulla and J. H. Schwab, *J. Bacteriol.* **91**, 374 (1966).
3. J. H. Schwab, *J. Bacteriol.* **90**, 1405 (1965).
4. G. E. Murphy and C. G. Becker, *Am. J. Pathol.* **48**, 931 (1966).
5. B. Sacks, *Amer. Heart J.* **1**, 750 (1925); G. E. Murphy, *Medicine* **39**, 289 (1960); ———, *ibid.* **42**, 73 (1963).
6. O. Saphir, *Amer. J. Clin. Pathol.* **31**, 534 (1959).
7. W. J. Cromartie and J. G. Craddock, in preparation.
8. We thank Mr. J. B. Taylor, Mrs. S. K. Anderle, and Mrs. P. S. Clark for technical assistance. Supported in part by USPHS Grant 5-01-AI05844. J. G. C. was supported in part by USPHS Training Grant 5-TO1-AI00045.

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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.