14854

Cold Hemagglutinins in Visceral Leishmaniasis (Kala-Azar).

HARRY M. ROSE. (Introduced by A. R. Dochez.)

From the Departments of Medicine and of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.

Cold agglutinins, or autoagglutinins, have been described in a wide variety of diseases including pneumonia, pernicious anemia, leukemia, hemolytic syndromes, hepatic cirrhosis, and peripheral vascular disorders.[1] It is evident, however, that such agglutinins do not occur regularly in any of these conditions, but rather sporadically and in an exceedingly haphazard manner. On the other hand it has long been known that autoagglutinins, or cold agglutinins, are commonly present in the blood of both humans and animals suffering from trypanosomiasis,[2] while more recent observations indicate that a similar serological response occurs frequently in cases of primary atypical pneumonia.[3,4,5]

The present report deals with the discovery of cold agglutinins in the blood of 2 patients suffering from visceral leishmaniasis (kala-azar). Apparently the only reference in the literature to a similar serological observation is Manson-Bahr's brief statement that autoagglutination occurs frequently in the disease.[6] Napier says that he has seen autoagglutination in kala-azar, but does not believe it to be of common occurrence.[7]

Case No. 1—S. T., a 15-year-old Filipino school girl, was admitted to the Presbyterian Hospital on July 20, 1943. One year before admission the patient emigrated to the United States from Calcutta, India, where she had previously resided for several years. Four months before admission she first noted symptoms of an illness which was characterized by remittent chills and fever, anorexia, progressive loss of weight, aching pain in the left upper quadrant, and gradual swelling of the abdomen. On physical examination the chief findings were pallor, emaciation, and a tremendously enlarged spleen. The significant laboratory data were as follows: erythrocytes 3,600,000, hemoglobin 8.6 g, leucocytes 2500, polymorphonuclear neutrophiles 53%, lymphocytes 34%, monocytes 13%, sedimentation rate 105 mm in one hour (Westergren), Wassermann anti-complementary, serum proteins 12.8 g% (albumin 3.6, globulins 9.2), cephalin flocculation test 4+, serum cholesterol 102 mg%. Microscopical examination of the sternal marrow revealed numerous Leishman-Donovan bodies. Chemotherapy was instituted with neostibosan intravenously, and a total of 3.5 g of the drug was administered over a period of 26 days. Clinical improvement was noted within a week after treatment was begun and thereafter the temperature remained normal, the patient gained weight, and the spleen decreased rapidly in size. At the time of discharge on August 31, 1943, 37 days after admission, the patient was completely asymptomatic, and 2 months later, on October 29, the spleen could not be palpated.

Case No. 2—Th., a 23-year-old Indian seaman, was admitted to the United States Marine Hospital at Ellis Island, N.Y., on August 16, 1943.* A diagnosis of kala-azar was made and confirmed by the finding of *Leishmania donovani* in material obtained by splenic puncture. The patient was successfully treated with neostibosan. On October 5, 1943, a specimen of blood was obtained through the courtesy of Dr. Harry Most, of New York University.

[1] Stats, D., and Wasserman, L. R., *Medicine*, 1943, **22**, 363.

[2] Yorke, W., *Ann. Trop. Med. and Parasitol.*, 1911, **4**, 529.

[3] Peterson, O. L., Ham, T. H., and Finland, M., *Science*, 1943, **97**, 167.

[4] Turner, J. C., Nisnewitz, S., Jackson, E. B., and Berney, R., *Lancet*, 1943, **1**, 765.

[5] Meiklejohn, G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 181.

[6] Manson-Bahr, P., *Manson's Tropical Diseases*, The Williams and Wilkins Co., Baltimore, 11th Edition, 1940, p. 191.

[7] Napier, L. E., personal communication.

* This case has been reported by Price, F. L., and Mayer, R. A., *J. A. M. A.*, 1944, **125**, 490.

TABLE I.
Titer of Cold Agglutinins for Group O Erythrocytes in Two Cases of Leishmaniasis.

Patient	Date	Serum dilutions 1-4	1-8	1-16	1-32	1-64	1-128	1-256	1-512	1-1024
S.T.	9-10-43	++++	++++	+++	++	++	+	0	0	0
	24	++++	++++	++++	+++	+++	+	0	0	0
	10- 1	++++	++++	++++	++	++	+	0	0	0
	29	++++	++++	++++	++++	+++	++	+	0	0
	12-17	+	±	0	0	0	0	0	0	0
Th.	10- 5-43	++++	++++	++++	++++	++++++++		++++	+++	+

Tests for cold agglutinins were performed on 5 occasions in patient S.T. and on the single specimen obtained from Th. No attempt was made to preserve a high agglutinin titer by removing the serum before the blood samples were initially cooled or chilled. However, in each instance after the clotted blood was received it was placed in an incubator at 37°C for one hour, followed by immediate centrifugation and withdrawal of the serum. Serial doubling dilutions of the serum were made in tubes containing 0.5 cc of 0.85% saline. To each tube of one series 0.5 cc of a 1.0% washed suspension of the patient's erythrocytes was added, while the tubes of the second series received 0.5 cc of a similar suspension of Group O cells. The racks were then placed in a refrigerator at 5°C overnight, and the results read immediately after removal from the cold by flipping the tubes and estimating the degree of agglutination with the naked eye. Four-plus agglutination was indicated by a tightly-packed disc of cells and one-plus agglutination by finely granular clumping. After the initial readings had been made the tubes were placed at room temperature (20-24°C) for an hour, and in each case the agglutination was observed to be completely dispelled.

The results of the tests are given in Table I and show that the sera of the patients with leishmaniasis contained cold agglutinins varying in titer from 1-128 to 1-1024. Separate data for the titers obtained with autologous and homologous erythrocytes are not given, since the values were essentially similar. Using the same technic more than 300 sera from normal subjects and from patients with a variety of other diseases were also examined, nearly all of which showed no cold agglutination whatsoever, although a small number showed weak agglutination at titers not exceeding 1-8.

Discussion. In patient S.T. the titer of cold agglutinins was found to be 1-128 on September 10, 1943, at which time the course of therapy with neostibosan had been completed and the clinical status was markedly improved, although the spleen was still very large. Seven weeks later, on October 29, when the patient appeared to be entirely well and the spleen could no longer be felt, the cold agglutinin titer was still undiminished. However, a subsequent examination on December 17 revealed that the cold agglutinins had almost completely disappeared from the blood, only a trace of agglutination being present at a serum dilution of 1-8. The association of the serological phenomenon with the acute phase of the illness, and its disappearance following the specific treatment and recovery of the patient, would apparently indicate that the cold agglutinins developed as the result of the infection and were not merely a chance finding. This conclusion is borne out by the discovery of similar antibodies in high titer in the second case of leishmaniasis.

The reasons for the development of cold agglutinins and their significance in leishmaniasis are unknown, and the incidence of their occurrence in the disease is yet to be determined. This report is made with the hope that investigators more favorably situated may cast further light on these problems.

Summary. Cold agglutinins in high titers were demonstrated in the blood of two patients suffering from visceral leishmaniasis. In one of these patients, who was followed for several months, the agglutinins returned to within normal limits after recovery.