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Blood Factors in the Nutrition of *Trypanosoma cruzi*[1]

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Although successful media for the *in vitro* culture of the blood parasite *Trypanosoma cruzi* invariably contain blood or blood derivatives, the nature of the essential blood factors remains ill defined. It appears likely from the work of numerous investigators that hemoglobin is one source of these factors (reviewed by M. Lwoff [*1*] and von Brand [*2*]). Little and his associates (*3–5*) have described media containing as their sole blood component a coagulum made from rabbit erythrocytes, or chick red cells applied on filter paper and autoclaved. Other writers (*6*) have stated that hemoglobin is not necessary. We have obtained satisfactory results using human red cell coagulum in conjunction with a basal medium of glucose, NaCl, and peptone in the concentrations recommended by Little and SubbaRow (*3*). Attempts to extract, concentrate, and identify the essential nutrient or nutrients from the solid coagulum have not been successful. The following experiments, however, have led us to the conclusion that the active principle for our strain of the parasite is a derivative of hemoglobin.

Thrice recrystallized hemoglobin was prepared from 6 times washed human erythrocytes by the method of Drabkin (*7*). The protein was then dissolved in water, dialyzed until essentially free of salts, and stored under sterile conditions after Seitz filtration. Cultures were carried out in a diphasic medium consisting of a 3-ml agar slant and a 2-ml liquid overlay. The basal medium containing 0.2% glucose, 0.5% NaCl, and 2% peptone at pH 7.4 was used in both the agar and liquid phase. Three to 4 mg of hemoglobin was added to the agar phase prior to autoclaving, the agar being used primarily as a solidifying agent. Each subculture was carried out in sextuplicate, using 0.1 ml inocula from the preceding culture, serial transfers being made at 18–22-day intervals, at which time the parasite count was about 12,000,000/ml.

Starting from a stock culture originally obtained from Costa Rica through the courtesy of Herbert Johnstone, of the University of California Medical Center, and grown by us on human red cell coagulum, agar, glucose, NaCl, and peptone, we have carried the organism through 13 serial transfers on the hemoglobin medium, with no diminution in rate of reproduction. Concurrent with the eighth serial subculture, a series of media containing graded amounts of hemoglobin from none to approximately 3 mg was inoculated. Growth responses in this series were essentially proportional to the amount of hemoglobin present. Substitution of hemin, of acid or peptic hydrolysates of hemoglobin, and of a heme-globin mixture for the heat-treated protein have yielded negative results. We were unable to maintain growth beyond the second subculture when the hemoglobin was not heated; however, the addition of ascorbic acid or of serum to unheated hemoglobin has resulted in positive responses to date through 5 and 8 subcultures, respectively.

On the basis of these observations it appears that a moderately complex derivative of hemoglobin is the only additional essential growth factor for our strain of *T. cruzi* when peptone, glucose, and NaCl are present in the medium. Work is in progress in an attempt to determine the nature of the active substances arising from the heat treatment of protein. As part of this investigation other heme-protein combinations are also being studied.

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of California.

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The Archaeological and Paleontological Salvage Program at the Medicine Creek Reservoir, Frontier County, Nebraska

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This report summarizes the results of scientific salvage operations during the past six years by the University of Nebraska State Museum at the Medicine Creek Reservoir. Medicine Creek is a major northern tributary of the Republican River in the dissected loess plains of southwestern Nebraska.

The Bureau of Reclamation completed work on the Medicine Creek Dam in 1949 as part of the Missouri Basin Development Program. Several archaeological and paleontological sites were destroyed in the course of construction work, and many more have been inundated by the Medicine Creek Reservoir, which reached normal pool level in 1951. Following the pattern set for reservoir projects throughout the Missouri Basin, a number of institutions participated in a salvage program aimed at the recovery of as much information as possible from these sites before they were destroyed.[1]

[1] There had been field work in the Medicine Creek Valley previous to the salvage investigations described here (*1–3*).