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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*).

The Missouri River Basin Survey of the Smithsonian Institution and the Nebraska State Historical Society conducted archaeological work, which has been reported elsewhere (*4*). The University of Nebraska State Museum undertook paleontological and geomorphological investigations, which later were accompanied by archaeological work.

Seven major localities have been intensively investigated by Museum parties.[2] Three of these sites are of late Pleistocene age, and four are late Pliocene.

The late Pleistocene localities proved to be of more than purely paleontological interest, since the faunal material in them was associated with evidences of human activity. These three sites are the Lime Creek Site (Ft-41), the Red Smoke Site (Ft-42), and the Allen Site (Ft-50).[3] All are in the lower part of the fill of Republican River Terrace-2A, tentatively dated as Mankato (Wisconsin-IV) (*5, 6*). The sites originally appeared as strata containing bone fragments, flint, and charcoal in the faces of vertical loess cliffs along Medicine Creek and its tributary Lime Creek. Excavation has revealed bowl-shaped fireplaces without stone lining, burned and broken bones, piles of flint chips, and occasional stone and bone artifacts, among which the most significant diagnostic items are projectile points. No human skeletal material, except for a single phalanx, has been recovered.

At the Lime Creek Site an upper stratum contained two points almost identical with certain of those illustrated by Krieger from the Plainview Site in Texas (*7*). Separated from this stratum by 8 ft of undisturbed silts was a lower occupation level, which has been reported previously (*8*). Parallel-sided points with straight bases came from this level. Charcoal obtained from 1 to 2 ft below the lower occupation level, but in the same sedimentary unit, has been given a radiocarbon date of 9524 ± 450 years (*9*).

The Red Smoke Site contains at least eight strata with artifacts and other evidences of human activity. From a middle layer has come a series of points and point fragments that correspond in form to Krieger's Plainview type (*10*). The other occupation levels have not yielded diagnostic material.

The Allen Site has been reported by Holder and Wike (*11*). They find that the artifact complex, which differs from those mentioned above, resembles most closely that found at Deadman Cave in Utah (*12*). Radiocarbon dates from the Allen Site are not consistent (*13*), but dates of 8274 ± 500 and 10,493 ± 1500 years for charcoal from the lower part of the occupation zone are felt by the present authors to be the most reliable ones in terms of the total evidence.

[2] This work was supported in part by funds from the following: Hector Maiben, Mr. and Mrs. Ben Maiben, and Childs Frick funds through the University of Nebraska Foundation; the University of Nebraska Research Council; the National Park Service; and the U. S. Bureau of Reclamation. The cooperation of the Missouri River Basin Survey of the Smithsonian Institution is also acknowledged.

[3] The code site designations used here are those employed by the Museum. According to the site designation system used by the Smithsonian Institution and several other organizations, these sites would be listed as 25FT41, 25FT42, and 25FT50, respectively.

It is impossible, so far, to determine temporal or cultural relationships between the archaeological manifestations found in these several sites, owing largely to the scarcity of diagnostic material. Sedimentary evidence indicates that the strata containing Plainview points at the Red Smoke and Lime Creek sites are approximately contemporaneous, and strengthens the typological conclusion that they are culturally related.

Judging from the evidence in these sites, southwestern Nebraska was occupied in late glacial times by wandering groups of bison hunters. Some of these groups, representing more than one cultural tradition, camped repeatedly along Medicine Creek—probably because of the abundance there of easily worked jasper and the presence of plentiful game. Further work is necessary before it will be possible to determine the local sequence of occupation. This information will then contribute to the broader story of the part played by human beings in the changing ecological situation on the Great Plains during late glacial and early postglacial times.

The paleontological material obtained from the above-mentioned late Pleistocene sites is of special interest because it represents the first extensive fauna from deposits of this age to be reported from the Great Plains region. Some of the faunal elements are similar to modern forms, whereas others appear to represent extinct species. The coyote bones, for example, cannot be differentiated from those of the modern species, *Canis latrans* Say, whereas the samples of bison compare favorably with those of the extinct form *Bison antiquus* Leidy from the late Pleistocene deposits of the Great Plains rather than with the modern *B. bison*. The radiocarbon dates obtained from two of the sites are a valuable aid in the study of the actual rate of evolution that has taken place during the past 10,000 years in a number of phylogenetic lines of mammals. Additional faunal evidence from the more recent terrace fills of the region has provided supplementary data on the rate and trends in evolution. These evolutionary yardsticks may well give us a clearer understanding of mammalian development in the earlier, as well as the later, part of the Pleistocene.

In the late Pliocene localities paleontological work took place primarily in the Kimball formation of the Ogallala group, which contained the remains of the shovel-tusked mastodont *Amebelodon fricki*, as well as various new species of mammals. The most important single specimen salvaged from the Kimball sands was the skull and ramus of a new form of giant saber-toothed cat, not ancestral to the saber-toothed felids of the Pleistocene. The Kimball formation heretofore had yielded only fragmentary faunal evidence in the central Great Plains. The Ash Hollow sediments directly below the Kimball also yielded fossils. The new material has helped to complete the late Pliocene faunal sequence and hence has thrown much new light on the Pliocene-Pleistocene boundary line problem.

An intensive geological and geomorphological study of the Medicine Creek area has accompanied the

archaeological and paleontological work. Detailed reports on all aspects of the Museum's Medicine Creek field project are in preparation.

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Antagonistic Action of Uracil, Thiouracil, and Thiourea on Reticulocyte Ripening

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Granulocytopenia and agranulocytosis are well-known complications of the treatment of thyrotoxicosis with the drugs of the thiourea group. A few cases of anemia have been observed (*1–3*). A slight drop in erythrocyte count has been found by Goldsmith, Gordon, Finkelstein, and Charipper (*4*) in rats poisoned by thiourea. According to Weinglass, Williams, Bissel, and Peters (*5*), a decrease of hemoglobin level is evidenced in rats receiving thiouracil. We have previously shown that thiourea, thiouracil, and methylthiouracil inhibit reticulocyte ripening *in vitro* (*6*). Besides, an antagonistic action of thiouracil and uracil on bacterial growth has been observed by Strandskov and Wyss (*7*) and Wolff and Karlin (*8*). On the basis of these observations, it appears quite possible that the inhibiting activity of thiouracil and its derivatives on blood cell ripening and regeneration could be due to an interference with the normal metabolism of uracil. This hypothesis seems to be confirmed, in the particular case of red blood cells, by the experiments described in this paper. The experimental results show a competitive antagonism of uracil, thiourea, and thiouracil on the ripening of young erythrocytes (reticulocytes) *in vitro*.

The observation of reticulocyte ripening *in vitro* has already made possible an experimental investigation of various toxic and metabolic factors concerned with erythropoiesis (*6, 9, 10*). Our technique for the study of reticulocyte ripening has been fully described elsewhere (*9, 11*); it will be summarized here.

[1] Fellow of the Belgian National Fund for Scientific Research.

Blood samples containing 10% heparin-glucose solution (heparin 200 mg %, glucose 1% in NaCl 0.9%) are drawn from adult dogs made anemic by repeated blood withdrawals, the hemoglobin level being maintained around 6–8 g %. The dogs are kept in good health and isolated; worms and other parasites are eliminated. Standard diet includes bread, 250 g; potatoes, 250 g; meat, 150 g; yeast extract, 2 g; and powdered iron, 40 mg daily. The animals submitted to chronic hemorrhage anemia show a high reticulocyte level in the peripheral blood (50–200/1000 RBC) and a normal reticulocyte ripening.

Basal solutions used are prepared as follows:

1)	Thiouracil	200 mg
	NaOH 1/10 *N*	17 ml
	Buffer at pH 7.34 (Na2HPO4 1/5 *M*-citric acid 1/10 *M*)	3 "
2)	Thiouracil	100 mg
	NaOH 1/10 *N*	7.5 ml
	Buffer at pH 7.34 (Na2HPO4 1/5 *M*-citric acid 1/10 *M*)	12.5 "
3)	Thiourea, 0.5% and 1% in NaCl 0.9%	
4)	Uracil, 5000, 500, 50, 25, 12.5, 5, 2.5 μg/ml NaCl 0.9%	

The above solutions are added to aliquot portions of the blood samples, in a proportion of 10–20%, in order to obtain concentrations of thiouracil or thiourea of 350–2000 μg/ml blood. The concentrations of uracil in the blood are between 500 and 0.25 μg/ml. Control experiments are performed without thiourea, thiouracil, or uracil supplementation. The blood samples are incubated at 37° C for 6–8 hr. Reticulocyte counts are made at the beginning and at the end of the incubation period, according to our usual technique (*12*) (observation on dark field of *dried* smears after post-vital staining with brilliant cresyl blue), in various parts of each smear, and on a minimum basis of 5000 RBC. Reticulocyte ripening is evidenced by the decrease in reticulocyte number during incubation of the blood. The higher the decrease, the faster the ripening of young erythrocytes. Experimental results are depicted in Tables 1 and 2.

The experiments demonstrate that reticulocyte ripening is slowed or blocked by either thiourea or thiouracil at a range of concentrations between 350 and 2000 μg/ml blood. The block of ripening is evidenced by a reduced decrease of reticulocyte number during incubation of the blood.

The block of ripening is lifted by uracil at considerably lower concentrations (less than 5 μg/ml). After simultaneous supplementation with thiouracil and uracil, or thiourea and uracil, the reticulocyte ripening is normal and proceeds at the same rate as in control experiments. A competitive antagonistic action of thiourea, thiouracil, and uracil on red cell ripening is evidenced.

On the basis of the above-described results it appears very probable that the inhibition of erythropoiesis by thiourea and thiouracil is due to an interference of these drugs with normal uracil metabolism. It should be remembered that uracil is a constituent