THE ROLE OF SULFUR IN THE METABOLISM OF *ENDAMOEBA HISTOLYTICA**

by

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Suspensions of *in vitro* cultures of *Endamoeba histolytica* produce CO_2 under anaerobic conditions from glucose, fructose, and mannose at equal rates and from sucrose and galactose at a lower rate in the presence of cysteine, with the simultaneous evolution of H_2S. It can be shown with amoeba cultures, starved for 14 hours in N_2 atmosphere, that the CO_2 and H_2S production are interdependent reactions, *i.e.* no H_2S is produced from cysteine unless hexose is added, and no CO_2 is produced from hexose unless cysteine is present. Parallel bacterial controls show that the bacteria necessary for the growth of amoebae in culture do not interfere with these metabolic measurements. With pyruvate as substrate the rate of CO_2 production by intact amoebae is two-thirds of that from glucose. When amoebae are quickly ground in a glass grinder, pyruvate becomes equivalent to hexose as substrate for CO_2 production. During the decarboxylation of pyruvate, however, no H_2S is formed from cysteine. When the cell structure of the amoebae is destroyed by quick freezing and thawing, no H_2S and CO_2 are formed with glucose and cysteine as substrates unless a heat-stable extract of rat liver acetone powder[1] is added, in which case the rate of gas evolution is equal to or greater than that from suspensions of intact starved cells. Control measurements on the bacteria present in amoeba cultures show that the reaction dependent on the liver coenzyme occurs exclusively in the protozoa. The liver coenzyme preparation cannot be replaced by coenzyme I, coenzyme II, diphosphothiamine, pyridoxine + adenosine triphosphate, or coenzyme A. NaH_2AsO_4 (10^{-3} *M*) inhibits CO_2 production from glucose but not the simultaneous H_2S formation from cysteine. A variety of chelating agents (8-hydroxyquinoline, α-α' bipyridyl, KCN, NaN_3, Chel 242L (Alrose Chemical Co.)) inhibit both CO_2 and H_2S production. Aged cytolyzed preparations, which lose the ability to catalyze CO_2 formation from glucose in the presence of cysteine and the liver coenzyme, are still able to use fructose-1,6-diphosphate as a substrate for CO_2 production and to support H_2S evolution. Such a preparation can decarboxylate pyruvate, but without simultaneous H_2S formation from cysteine.

These experiments suggest that *Endamoeba histolytica* contains a triose phosphate oxidase which transfers hydrogen to the sulfur atom of cysteine. A metallo-protein enzyme and a cofactor (or cofactors) present in the liver coenzyme preparation are constituents of this catalytic system. The molar ratio of CO_2 and H_2S varies between 10 to 20 moles of CO_2 to 1 mole of H_2S. The rate of formation of H_2S is considered as a measure of the efficiency of the oxidation of triose phosphate which is coupled with desulfuration of cysteine. In the course of this reaction thiol radicals[2] are likely to be formed which either react with other intermediates or, depending on conditions, appear as H_2S. The participation of thiol radicals is suggested by the strong inhibitory action of certain semiquinones (0.3 μM pyocyanine per ml causes complete inhibition). The reaction can be directed towards H_2S production by small amounts (0.03 μM per ml) of 2,3-diaminophenazine. Previous observations[3] demonstrated that optimal growth of *Endamoeba histolytica* occurs when the E^h of the cysteine-containing medium is between —120 and —145 millivolts. This E^h value agrees closely with that of sulfur between the oxidation states of 0 and —2, *i.e.* —141 millivolts[4].

Since it is known that other microorganisms exhibit stimulation of H_2S production upon addition of carbohydrates[5], it is possible that the type of biological oxidation observed in cultures of amoebae is of a more general nature. Further investigations will show whether or not the desulfhydrase of mammalian liver is a part of a similar catalytic system.

A detailed account of this work will be published later.

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ON THE POLYSACCHARIDE OF FIBRINOGEN AND FIBRIN

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It has been observed in the course of amino acid analysis of fibrin hydrolysates that a very marked Molisch reaction could be obtained. In connection with this observation fibrinogen of high purity was prepared from bovine plasma and from plasma of other mammalians by a modified method of our own[1]. Conversion of fibrinogen into fibrin was carried out with bovine thrombin (Hoffman la Roche and Richter) under special conditions[2] in order to facilitate elimination of other protein molecules and of non-protein contaminants. The clot was washed with distilled water, ethanol and ether, and dried *in vacuo* over $CaCl_2$ to constant weight.

For paper chromatography, the fibrin thus prepared was hydrolysed in a water bath at 100° C with $N/2$ H_2SO_4 for 6 to 8 hours. After neutralising with an aqueous solution of barium hydroxide, the resulting $BaSO_4$ was filtered, the filtrate was evaporated *in vacuo*, and the residue dissolved in a small amount of water was subjected to chromatography. Chromatograms were run on Macheray-Nagel paper No. 214 according to the method of PARTRIDGE[3] with butanol-acetic acid and dried. After repeating this process the sugars were located with anisidine phosphate[4].

Three reducing sugars were identified, the spots corresponding to mannose, galactose and glucosamine, respectively[5]. The same three reducing sugars were identified in chromatographic examinations of fibrins of other mammalians (man, horse, sheep and rabbit).

Fibrinogens of different species have also been studied with chromatography. The solutions of fibrinogens containing 95–96% of clottable protein were freed from salts by dialysis against distilled water. The precipitated fibrinogens were washed, dried, and hydrolysed by the method described above. Chromatography showed the presence of the same three sugars as in the case of fibrin.

Although chromatography had shown no qualitative difference between the polysaccharides of fibrinogen and fibrin, the quantitative determination of hexose[6,7] and glucosamine[7] has revealed marked differences between the corresponding fibrinogen and fibrin of all species studied.

TABLE I

HEXOSE AND GLUCOSAMINE CONTENT IN % OF DRY WEIGHT

	n+ *Hexose* %	*n*+ *Glucosamine* %
Bovine fibrinogen	4 1.64 ± 0.02*	2 0.56 ± 0.02*
Bovine fibrin	4 1.33 ± 0.04	2 0.54 ± 0.01
Rabbit fibrinogen	4 1.98 ± 0.03	2 0.59 ± 0.03
Rabbit fibrin	4 1.66 ± 0.02	2 0.60 ± 0.02

n+ No. of determinations; * Standard deviation