Effect of Sulfonamides on Thiamine Requirement of *Tetrahymena geleii* (H). (20930)

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Kratzing and Slater have reported that the inclusion of sulfadiazine in a diet low in thiamine prevented the development of typical thiamine-deficient symptoms in the rat(1). They concluded that sulfadiazine has a true thiamine-sparing action on the animal. Preliminary studies in this laboratory(2) have indicated a similar condition in the ciliated protozoan, *Tetrahymena geleii*. The present paper is a first report of observations on the effect of sulfadiazine and other sulfonamides on the thiamine requirement of *Tetrahymena*.

Methods. The ciliated protozoan, *Tetrahymena geleii* (H), grown in pure culture, was employed in this investigation. The base medium was essentially that of Dewey *et al.*(3), modified as indicated in Table I. The organisms were grown in 50 ml Erlenmeyer flasks containing 5 ml of medium, according to the technic described by Hutner(4). All inoculations were made through a dilution flask containing only basal medium. The flasks were kept at 25°C, and the cultures were harvested on the 10th day following inoculation. Growth of the harvested cultures was measured by cell counts, using a Sedgewick-Rafter counting chamber and Whipple ocular micrometer, as described by Hall *et al.*(5).

TABLE I. Composition of Basal Medium.*

	ml %
Solution B (amino acids)	14.0
C (vitamins) †	6.0
D (salts)	2.5
E (salts)	0.6
F (phosphates)	0.175
J (purines and pyrimidines)	5.0
	%
Glucose‡	0.5
Sodium acetate	0.2
Protogen§	2.0
Tween 80	2.4
(pH 7.0)	

* The composition of the solutions listed here are identical with those listed by Dewey *et al.*(3, p. 284).

† Thiamine omitted in appropriate experiments.

‡ Autoclaved separately and added aseptically.

§ Units/ml.

TABLE II. Response of *T. geleii* to Sulfadiazine.

Sulfadiazine (mg %)	No thiamine, cells/ml (×10³)	pH	Thiamine (0.1 mg %), cells/ml (×10³)	pH
0	Dead*	4.5	468	6.8
.1	2	5.0	478	6.8
1	92	6.8	590	6.8
5	194	6.8	540	6.8
10	265	6.8	528	6.8
12	182	6.8	498	6.8

* No viable cells on the 10th day.

Results. Table II shows the results of a typical experiment with sulfadiazine. It is apparent from this data that cultures containing the optimum quantity of sulfadiazine (10 mg %) without thiamine support growth of *Tetrahymena* but not as well as optimum concentrations of thiamine do. This is true to a lesser extent for series at concentrations of 5.0 mg % and 12 mg % sulfadiazine. Furthermore, control cultures containing neither thiamine nor sulfadiazine but complete in every other respect show a rapid drop in pH. Microscopic examination of these latter cultures fail to demonstrate the presence of living cells and chemical analysis reveals that an accumulation of pyruvic acid has occurred. This observation is rather typical of thiamine-deficient cultures(6).

A large number of experiments with other sulfonamides: sulfamerazine, sulfathiazole, sulfanilamide and sulfapyridine did not have a thiamine-sparing action.

Summary. It has been found that the inclusion of 5.0 mg % to 12 mg % sulfadiazine in a complete culture medium minus thiamine, supports growth of the ciliate, *Tetrahymena geleii*. At optimum concentrations this growth is about 50% of that in medium with added thiamine. The optimum concentration appears to be 10 mg % sulfadiazine. Cultures containing neither sulfadiazine nor

thiamine but complete in every other respect show a drop in pH and an accumulation of pyruvate. Other sulfonamides gave negative results. At the present time no hypothesis is offered to account for this phenomenon pending further investigations.

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Dicumarol and Plasma Antithrombin Activity.* (20931)

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Recent advances in the subject of prothrombin activation have brought changes in our ideas concerning the effect of dicumarol on the blood coagulation mechanisms. Dicumarol is no longer considered to act exclusively with respect to prothrombin, but also in relationship to the activation of prothrombin. The inhibitors of the blood clotting mechanisms may possibly also be involved. This paper is an attempt to contribute to the subject of antithrombin levels in dicumarol plasma which is a problem that still remains a matter of uncertainty. Hurn, Barker, and Mann(1) reported an increase of antithrombin activity in serum after dicumarol therapy. Whether this represents a real antithrombin increase or is the result of unknown factors which influenced their antithrombin test has not been completely clarified. Owen and Bollman found that dog plasma exerts about the same antithrombic activity regardless of the prothrombin concentration after dicumarolization(2). At the time their papers were written less was known about the fundamental nature of plasma antiprothrombin activity than today. The background of our paper is based on the idea that more than one mechanism is involved in antithrombin activity, and that it is possible to analyze the diverse aspects of thrombin inactivation in separate systems of study. For a better understanding of the problem the following nomenclature classifying the diverse antithrombin reactions is used in this laboratory. Antithrombin-I refers to the adsorption of thrombin on fibrin. Antithrombin-II concerns the heparin cofactor, which together with heparin interferes with the interaction of thrombin and fibrinogen. Antithrombin-III refers to the natural antithrombin, which neutralizes thrombin quantitatively as a function of the thrombin concentration, and does not require heparin. Antithrombin-IV seems to be independent of the actions cited above, and becomes manifest during the process of prothrombin conversion. It is the activity which remains after antithrombin-III activity is removed by ether extraction. We want to emphasize that this is an arbitrary nomenclature, and in part based on theory, since the experiments performed in this field do not provide conclusive support for the existence of 3 independent substances. It is still possible to consider the antithrombin-IV phenomenon as interactions of different and dissociable phenomena arising from the ar-

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