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Replacement of Ascitic Fluid or Rabbit Serum Requirement of *Treponema dentium* by α-Globulin

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Three strains of *Treponema dentium* were isolated in a veal heart infusion medium enriched with 10% ascitic fluid prepared according to the method of R. J. Fitzgerald and E. G. Hampp (J. Dental Res. **21**:20, 1952). These organisms had a "2-4-2" axial fibril relationship by electron microscopy (M. A. Listgarten and S. S. Socransky, Arch. Oral Biol. **10**:127, 1965) and did not ferment carbohydrates or utilize glucose or lactate. The organisms produced acetic acid and ammonia as major end products in the veal heart infusion-ascitic fluid medium.

A number of commercially available culture media were used in an attempt to replace the veal heart infusion base. The best basal medium was a medium used for the cultivation of another oral spirochete, *T. microdentium*. This medium consisted of P P L O Enrichment Broth (BBL) without crystal violet or serum, supplemented with (per milliliter): 1 mg of glucose, 400 μg of nicotinamide, 150 μg of spermine tetrahydrochloride, and 20 μg of sodium isobutyrate. The *p*H was adjusted to 7.5 with sodium hydroxide and autoclaved at 121 C for 15 min. To this medium, filter-sterilized L-cysteine and cocarboxylase were added aseptically to a final concentration of 1 mg/ml and 5 μg/ml, respectively. When the above medium was used to replace veal heart infusion, it was found that *T. dentium* still had an absolute requirement for ascitic fluid or rabbit serum. Maximal growth occurred when the fluid enrichments were present in a 10 to 20% concentration.

To determine the active components present in ascitic fluid or rabbit serum, the supplemented P P L O medium was used as a base, and the various fractions to be assayed were added aseptically after filter sterilization. The organisms were grown for 2 to 3 days at 35 C in an atmosphere of 95% H_2 and 5% CO_2. Turbidity was determined by nephelometry on the sixth serial transfer of the organisms in the test medium.

Preliminary characterization of the active factor indicated that it could not be ashed, and it was inactivated by heating at 80 C for 30 min; it was stable in visible, infrared and in ultraviolet light; it was not dialyzable; it was not volatile or steam-distillable at acidic, neutral, or basic *p*H, it would not partition from water into ether at acidic, neutral or basic *p*H, and it was excluded by Sephadex G-25, G-50 and G-75 gels, but not by Sephadex G-100 or G-200. The factor passed through a column of Dowex 50 W in the H^+ cycle and could not be eluted from a column of Dowex 2 in the formate cycle.

TABLE 1. *Ability of certain serum protein fractions to support growth of Treponema dentium*

Protein fraction	Source[a]	Range of concn (%)	Growth relative to ascitic fluid medium[b]
Albumin (rabbit)	3191	0.05-2.0	8
Albumin, Fraction V (rabbit)	2117	0.05-2.0	9
β-Globulin, Fraction III (rabbit)	767	0.05-0.4	20
γ-Globulin, Fraction II (human)	457	0.07-0.4	2
Hemoglobin (bovine)	7082	0.01-0.2	4
α-Globulin, Fraction IV (rabbit)	6729	0.1-0.4	110
α-Globulin, Fraction IV (human)	157	0.0001-0.4	460
α_2-Globulin (human)	5127	0.0001-0.4	270
Transferrin (human)	368	0.001-0.1	12
Caeruloplasmin (bovine)	5851	0.0006-00.2	9

[a] Numbers refer to catalogue numbers for the Mann Research Laboratories.

[b] Best growth obtained at any concentration on the sixth serial transfer, expressed as a percentage of growth reached in a medium containing 10% ascitic fluid.

On attempting to purify the factor, it was found that the active factor was precipitated by ammonium sulfate at 33 to 50% concentration. By use of the Cohn method of ethyl alcohol precipitation (Cohn et al., J. Am. Chem. Soc.

68:459, 1946), most of the activity was found in Cohn Fraction IV. Acetone precipitation inactivated the factor. On Sephadex G-200 column chromatography (J. Killander, Biochim. Biophys. Acta 93:1, 1964), the active factor migrated immediately after the excluded volume, but well before the albumin or transferrin peaks.

A number of serum protein fractions were assayed on the basis of the above results to determine their ability to support spirochetal growth. Of these, commercially available Cohn Fraction IV (Mann Research Laboratories, Inc., New York, N.Y.) and α_2-globulin (Mann Research Laboratories) were most effective in supporting growth. Table 1 indicates the ability of certain protein fractions to support spirochetal growth in various concentrations, including those fractions which might be provided by 10% rabbit serum.

Concentrations of α_2-globulin or Cohn Fraction IV as low as 0.005% supported consistently greater growth of the three strains of *T. dentium* than did 10% ascitic fluid or rabbit serum through 18 serial transfers, after which the experiment was discontinued.

Optimal growth occurred at a final α_2-globulin or Fraction IV concentration of 0.05%; after 3 days of incubation, microscopic counts averaged 2.7×10^8 and 4.6×10^8/ml in the respective media.

The supplemented P P L O medium with α_2-globulin or Cohn Fraction IV is simple to prepare, is reproducible, and supports more abundant growth of *T. dentium* than the veal heart infusion-ascitic fluid medium.

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