Sensitivity Studies *in vitro* with *Histoplasma capsulatum* to Various Potential Therapeutic Agents. (18781)

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During recent years histoplasmosis as a significant clinical entity has been brought to the fore. The infection usually appears in the benign form, however, the fulminating type of the disease is more important as a clinical entity. Many drugs and antibiotics have been used in the attempt to treat this infection but without avail.

Material and methods. On February 21, 1949 an infant with histoplasmosis was admitted to the Children's Hospital. The patient was a 6½-months-old white female whose chief complaints were fever, cough and enlarged abdomen. The child had been hospitalized previously in another city and was referred to us with the diagnosis of acute lymphatic leukemia. A bone marrow biopsy was performed and the presence of intracellular yeastlike cells in the macrophages led to the diagnosis of histoplasmosis. Cultures of blood and bone marrow yielded *Histoplasma capsulatum*. Penicillin, aureomycin and sulfadiazine were administered, and general supportive therapy including repeated transfusions was instituted. The patient expired approximately 2 months after onset of the illness, which was 2 weeks after admission to the hospital. The organism isolated from the bone marrow of this patient was maintained in the yeast phase by frequent transfer on blood agar slants. Since the organism occurs in the infection in the yeast form, it is necessary that inhibition studies be conducted with this phase. By transferring at weekly intervals the culture grew rapidly, producing heavy growth within four days. *In vitro* studies were conducted using the gamma and delta isomers of benzine hexachloride, quinine bisulfate, atabrine, emetine, aminopterin, 2,4-dichlorophenoxyacetate, para-aminosalicylic acid, urethane, and ethyl vanillate. The benzine hexachloride isomers, aminopterin, urethane and para-aminosalicylic acid were incorporated in graded concentrations in blood agar using 5% human blood in the agar base described by Casman(1). The remainder of the materials were tested in a liquid medium described by Salvin(2). Although this medium contains no blood or serum, the organism is maintained in the yeast phase. The cultures were inoculated with a light suspension of *Histoplasma capsulatum*. The number of viable cells inoculated was not determined since the size inoculum did not significantly alter the sensitivity results. All cultures were sealed with parofilm to maintain the moisture and were incubated at 37°C. The first readings were made after 5 days incubation and final observations were made when the tests were discarded after 3 weeks.

TABLE I. Sensitivity Studies *in vitro* with *Histoplasma capsulatum* to Various Potential Therapeutic Agents.

Test material	Inhibited by, mg %	Tolerated, mg %
Gamma benzine hexachloride	10	5
Delta " "	10	5
Quinine bisulfate		3.2
Atabrine	3.3	.33
Emetine	3.2	.32
2, 4 dichlorophenoxyacetate		100
Para-aminosalicylic acid		200
Aminopterin		.1
Urethane		100
Ethyl vanillate*	50	40

* Obtained from The Institute of Paper Chemistry, Appleton, Wisc.

Results. The results of the *in vitro* studies are summarized in Table I.

The isomers of benzine hexachloride inhibited the test organism in a concentration of 10 mg %. These materials, known primarily as insecticides(3) have been found inhibitory for certain micro-organisms, including various yeasts and the tubercle bacillus, however, their

1. Casman, E. P., *Am. J. Clin. Path.*, 1947, v17, 281.

2. Salvin, S. B., *J. Bact.*, 1947, v54, 655.

3. Slade, R. E., *Chemistry and Industry*, 1945, v70, 314.

use as a potential therapeutic agent is quite limited because of the toxic properties. When mixed with blood or plasma these isomers are bound to the protein and have been found to be without value in the treatment of experimental tuberculosis in mice and guinea pigs(4).

Quinine bisulfate, used in the treatment of malaria, failed to inhibit growth in a concentration of 3.2 mg %, the maximum concentration tested.

Atabrine, another antimalarial drug, prevented the growth of the organism in a concentration of 3.3 mg %. This drug is deposited in high concentrations in the reticuloendothelial system and as reported elsewhere, has been active *in vitro* against *Histoplasma capsulatum*. Saslaw, Campbell, and Kuhn(5) reported that when administered early in the infection in mice, the mortality was reduced, however, atabrine was not effective once the disease was well established.

Emetine exhibited the same inhibitory concentrations as atabrine. This drug as used in the treatment of amebiasis is quite toxic to the myocardium, hence the potential application therapeutically is limited.

Because of its ring structure 2,4-dichlorophenoxyacetate (2,4-D), a selective herbicide(6), was included in the test materials. There was no inhibition at a concentration of 100 mg %.

Growth of *Histoplasma capsulatum* was not inhibited by para-aminosalicylic acid in a concentration of 200 mg %. Although this compound has bactericidal activity against *Mycobacterium tuberculosis*, its primary value has been the retardation of the development of streptomycin resistance in the tubercle bacillus(7).

Aminopterin, a biologic folic acid antagonist, failed to inhibit growth in the maximum concentration tested, 0.10 mg %. This concentration is higher than the level obtainable in the body.

Urethane, which is known for its anesthetic and growth suppressive properties, has been studied as a possible therapeutic agent for leukemia. This drug failed to suppress growth in a concentration of 100 mg %.

Ethyl vanillate, inhibitory in a concentration of 50 mg %, appeared to be the most promising material tested for the treatment of histoplasmosis. This substance is a by-product in the manufacture of sulfite pulp. Although it is relatively toxic, therapeutic levels can be obtained. Christie and co-workers(8) in a recent report stated that they used this drug in the treatment of 12 patients with the progressive type of histoplasmosis. Five of their patients recovered. Since this form of the disease is usually fatal and some of their patients had well advanced infections, the authors considered the recovery rate to be of significance. Ethyl vanillate was administered as a 40% solution in olive oil with a maximum dosage of 1.5 g per kg body weight per day. A blood level of 20 to 30 mg per 100 ml was usually obtained on this dosage. On the basis of our findings, their organisms were more sensitive than the strain which we studied. At the same time, ethyl vanillate was the only agent studied which showed promise as a possible therapeutic agent.

Summary. *Histoplasma capsulatum* isolated from an infant who succumbed to the infection was tested *in vitro* in the yeast phase to various substances known to be harmful to certain protoplasmic structures. The test materials were gamma benzine hexachloride, delta benzine hexachloride, quinine bisulfate, atabrine, emetine, 2,4-dichlorophenoxyacetate, aminopterin, para-aminosalicylic acid, urethane and ethyl vanillate. Growth was inhibited by ethyl vanillate in concentrations therapeutically attainable in man.

4. Fromageot, C. and Sejourne, Therese, *Biochem. and Biophys. Acta.*, 1948, v2, 478.

5. Saslaw, S., Campbell, C. C., and Kuhn, L. R., *Abst. Proc. 50th Meeting Soc. Am. Bact.*, 1950, 110-111.

6. Bein, M. and Schopfer, W. H., *Experientia*, 1948, v4, 222.

7. Graessle, O. E., and Pietrowski, J. J., *J. Bact.*, 1949, v57, 459.

8. Christie, A., Middleton, J. G., Peterson, J. C. and McVickar, D. L., *Pediatrics*, 1951, v7, 7.

Received June 5, 1951. P.S.E.B.M., 1951, v77.