out from the gonad toward the neighboring adrenal tissue.

5. Mesonephric differentiation both anterior and posterior to the gonad: In one embryo (120 hours) 12-15 mesonephric tubules appear together with 2 renal corpuscles. As all of the mesonephric differentiation occurs anterior and posterior to the gonad, the sex gland is again "isolated". Rete strands are clearly present and project toward the adrenal cortex.

In assessing these 22 cases showing mesonephric differentiation on the operated side, it should be noted that in all, a normal gonad with a full complement of rate strands developed in conjunction with an extremely small number of mesonephric tubules. The number of mesonephric tubules varied from 1-15. Now, if the mesonephric tubules or the renal corpuscles are the material source of the rete, then quantitatively there should be a corresponding deficiency of the rete. On the contrary, the rete on the operated side is always comparable to its counterpart on the unoperated side. Further, only 6 of the 22 cases have any renal corpuscles whatsoever on the operated side, yet rete strands are seen to develop and differentiate at a normal rate in all. These results, therefore, militate against a tubular or capsular origin for the rete.

Summary. The primordial rete of the chick embryo is a product of the gonadal blastema. It differentiates coincidentally with the primary sex cords with which it is continuous from the beginning. The rete testis is elaborated into a well vascularized network which invades the anterior portion of the mesonephros. There the rete tissues invest and ultimately fuse with the capsules of renal corpuscles. Cavitation of the rete testis begins within the first 3 days after hatching and is well advanced by the end of the 3rd week, when lumina appear in the sex cords. The rete ovarii in general parallels the development of the rete testis. However, its bulk is always less and shows only incipient cavitation. As a result of breakdown of the medullary cords accompanied by a disintegration of the connection between rete and renal capsules, the rete ovarii ends up as a rudiment isolated in the mesovarium. In embryos where a complete block of one mesonephros is effected experimentally, rete cords make their appearance associated and continuous with the sex cords of a normally differentiating gonad. In these cases, rete differentiation is independent of mesonephric differentiation. Embryos with the mesonephros only partially blocked on one side give evidence less critical but favorable to the view of gonadal origin of the rete.

1. Willier, B. H., Allen, *Sex and Internal Secretions,* 2nd edit., Williams and Wilkins Co., 1939, Chap. III, 64.

2. Torrey, T. W., *Am. J. Anat.,* 1947, v81, 139.

3. Gruenwald, P., *Arch. f. Entw.-mechan. d. Org.,* 1937, v136, 786.

4. Boyden, E. A., PROC. SOC. EXP. BIOL. AND MED., 1927, v24, 572.

5. Waddington, C. H., *J. Exp. Biol.,* 1938, v15, 371.

6. Mossman, H. W., *Anat. Rec.,* 1946, v94, 484.

7. Duke, K. L., *Anat. Rec.,* 1952, v112, 737.

Received July 22, 1953. P.S.E.B.M., 1953, v84.

A Comparison of Fungistatic Properties of Three Aromatic Diamidines. (20547)

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The introduction of the aromatic diamidines as hypoglycemia-producing agents and the demonstration of their chemotherapeutic activity in protozoal diseases has led to experimental studies on their activity in the fields of bacterial, mycological, and neoplastic diseases. Favorable clinical results in patients suffering from blastomycosis(1,2), actinomycosis(3), and nocardiosis(2) have been reported following treatment with stilbamidine.

Observations following treatment with the aromatic diamidines in histoplasmosis(4-7), torulosis(8), and coccidiomycosis(9) have been variable. Studies on the *in vitro* effectiveness of the aromatic diamidines have been limited(1,10-12). Further studies of the specificities of the diamidines against certain pathogenic fungi might help to explain some of the discrepancies in the clinical observations.

Methods. Sabouraud's glucose agar was prepared with distilled water and autoclaved for 15 minutes at 15 lb pressure. The agar was cooled to 45°C and appropriate amounts of Seitz filtered stilbamidine (4,4' stilbenedicarboxamidine), propamidine (p,p'-(trimethylenedioxy) (dibenzamidine) and pentamidine (p,p'-(pentamethylenedioxy) dibenzamidine)* were added. The concentrated aqueous solutions of the 3 drugs were Seitz filtered to sterilize, in view of the fact that in solution stilbamidine can be heated to 70-80°C for only a short time without decomposition(13) and it was felt that the chemical structures of the other 2 drugs were also prone to ready decomposition. The effect of amounts of 100 μg, 10 μg, and 1 μg of drug per ml of agar on the various fungi were tested. To obviate the effect of pH as noted by Elson(12), the pHs of different lots of the agar were adjusted to 7.4 following the addition of the diamidines. The fungi to be tested were initially grown on Sabouraud's glucose agar pH 7.5 for 28 days. The mycelial mass was removed aseptically with as little of the agar as possible and placed in a tube containing glass beads and 10 ml of sterile saline. This mixture of fungi and saline was shaken for 20 minutes in a Kahn shaker and 0.1 ml amounts of the mycelial-spore mixture were spread evenly over the surface of the agar slant containing the drug. The tubes were incubated in the dark for 30 days at room temperature and readings for inhibition were made at 4-day intervals. All of the *in vitro* tests were performed in triplicate and the presence of growth was determined by the use of the dissecting microscope and cotton blue mounts. *Candida albicans, Cryptococcus neoformans, Histoplasma capsulatum, Blastomyces dermatitidis, Microsporum audouini, Microsporum canis, Tricophyton rubrum, Tricophyton tonsurans* (crateriforme) *Sporotrichum schenkii, Coccidioides immitis,* and a Madurella species were employed as test organisms.

The results of the *in vitro* tests are shown in Table I.

Our results with stilbamidine and *H. capsulatum* closely paralleled the observations of Seaburg(10) but *in vitro* the 0.01 mg/ml concentration of propamidine had some inhibitory effect suggesting it may be the drug of choice. In the case of *C. neoformans* and propamidine we found a close correlation with the value as determined by Fisher(4) for inhibition but pentamidine at the 0.1 mg/ml level displayed complete inhibition. Although our figures for the fungistatic properties of propamidine against *M. audouini, M. canis, T. rubrum, B. dermatitidis, C. albicans,* and *S. schenkii* do not correspond exactly with those of Elson (12), it was felt that the minor variations could be explained on the basis of pH and strain differences. It appears from Table I that the drugs differ in their capacity to inhibit certain fungi *in vitro.*

In view of our findings and the previously reported observations, it was decided to determine the effectiveness of the 3 diamidines against *Sporotrichum schenkii* infection in mice. This organism was chosen because of a slow progression of the disease with the production of grossly recognizable nodules. Twenty-one day-old Carworth white mice weighing between 22 and 25 g were inoculated intraperitoneally with 0.2 ml of a gastric mucin(13) suspension of *Sporotrichum schenkii* (BJC '52) grown at room temperature on Sabouraud's glucose agar. The mice were maintained on normal ration until the 26th day post infection at which time all mice showing gross subcutaneous nodules were isolated and separated into 4 groups. Groups 1 through 4 received stilbamidine, propamidine, pentamidine, and no therapy respectively. Three small groups of normal uninoculated mice served as controls on the effect of the diamidines alone. The drugs were in-

* We are indebted to Dr. Stephen Fromer, Merck & Co., and to Dr. R. C. Pogge, Wm. S. Merrell Co. for supplying the drugs used in this investigation.

TABLE I. A Comparison of the *In Vitro* Fungistatic Activity of 3 Aromatic Diamidines against Pathogenic Fungi.*

	Stilbamidine, mg/ml			Propamidine, mg/ml			Pentamidine, mg/ml			
	.1	.01	.001	.1	.01	.001	.1	.01	.001	Control
C. albicans	1+	2+	4+	2+	3+	4+	1+	3+	4+	4+
C. neoformans	4+	3+	4+	1+	4+	4+	0	4+	4+	"
H. capsulatum	0	4+	4+	0	2+	4+	4+	4+	4+	"
B. dermatitidis	0	0	2+	0	1+	3+	0	2+	4+	"
S. schenkii (BJC52)	0	1+	4+	0	0	2+	0	0	2+	"
M. audouini	4+	4+	4+	0	4+	4+	0	0	4+	"
M. canis	4+	4+	4+	4+	4+	4+	4+	4+	4+	"
T. rubrum	1+	3+	4+	0	0	4+	1+	2+	3+	"
T. crateriforme	3+	4+	4+	1+	2+	4+	2+	3+	4+	"
C. immitis	0	2+	4+	0	4+	4+	0	2+	3+	"
Madurella sp.	0	4+	4+	0	2+	4+	0	2+	4+	"

* 0 = complete inhibition; 4+ growth comparable to that of the controls.

corporated into the drinking water of the mice and were made up fresh daily in distilled water to a final concentration of 0.2 mg/ml. Observations indicated that groups of 4 mice ingest between 22-23 ml of water/day, thus it was assumed that each mouse would average 1.0-1.6 mg of drug intake in a 24-hour period. The mice were maintained on the drugs for a 20-day period and then observed for an additional 30 days. It was presumed that any evidence of therapeutic success would be accompanied by a remission of the subcutaneous abscesses, thus mice surviving to the end of the 76th day with no remission of the nodules were considered to be still actively infected. Mice dying on or before the 76th day were autopsied and cultures obtained from one or more of the abscesses. The results from a group of 175 mice failed to show any clear statistical evidence of therapeutic improvement. Mortality in the control groups was 13.8% and in the 3 groups on diamidine therapy ranged from 10.0 to 15.8%. Histological sections on the liver and kidneys of random selected surviving mice showed only slight degenerative changes† attributed to the increased toxicity of the diamidines which were exposed to light for 12-16 hours in the water bottles.

Discussion. The diamidine spectrum of 7 of the fungi causing systemic mycosis and 4 causing dermatomycosis have been examined. In general these findings have indicated some correlation with values obtained by other investigators. These figures indicate quantitative differences in the degree of inhibition of the 3 aromatic diamidines *in vitro*. Thus, the failure of certain of the diamidines to influence the clinical course of torulosis may be due in part to the relative ineffectiveness of the specific drug *in vitro* at levels that can be achieved in the blood stream. If these observations can be substantiated by other workers using different strains of the same fungi, it may become necessary to determine the drug sensitivities *in vitro* prior to instituting treatment.

The failure of the 3 aromatic diamidines to influence the course of *Sporotrichum schenkii* infection in mice may be due to an inability to achieve a sufficiently high level of the drug in the blood stream. This preliminary experiment was an effort to determine the effect of the drugs on sporotrichosis in mice, the possibility of an easier route of administration and the effect of maintaining a constant level of drug in the blood stream.

Summary. 1. The *in vitro* sensitivity of 11 fungi have been found to differ appreciably in the presence of stilbamidine, propamidine, and pentamidine. 2. The 3 diamidines appeared to have no therapeutic effect upon sporotrichosis in mice under the conditions of the experiment.

† The author is indebted to Dr. Melvin Haley, Dept. of Pathology, Baylor, for examination of the histological sections of the experimental mice.

1. Curtis, A. C., and Harrell, E. R., *Arch. Dermat. and Syph.*, 1952, v66, 676.

2. Schoenbach, E. B., Miller, J. M. Ginsberg, M.,

and Long, P. H., *J.A.M.A.*, 1951, v146, 1317.
3. Miller, J. M., Long, P. H., and Schoenbach, E. B., *J.A.M.A.*, 1952, v150, 35.
4. Seabury, J. H., *Ann. Int. Med.*, 1949, v31, 520.
5. Parsons, R. J., and Zarafonetis, C. J. D., *Arch. Int. Med.*, 1945, v75, 1.
6. Ellis, F. F., Scott, R. J., and Miller, J. M., *Antibiotics and Chemotherapy*, 1952, v2, 347.
7. Charr, R., *Am. Rev. Tb*, 1953, v67, 375.
8. Miller, J. M., Schoenbach, E. B., Long, P. H., Shuttleworth, J. S., and Snider, G. E., *Antibiotics and Chemotherapy*, 1952, v2, 444.
9. Cohen, R., *J.A.M.A.*, 1952, v150, 322.
10. Seabury, J. H., and Artis, D., PROC. SOC. EXP. BIOL. AND MED., 1946, v61, 15.
11. Fisher, A. M., *Bull. Johns Hopkins Hosp.*, 1950, v86, 383.
12. Elson, W. O., *J. Infect. Dis.*, 1945, v76, 193.
13. Campbell, C. C., and Saslaw, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 469.

Received July 27, 1953. P.S.E.B.M., 1953, v84.

Failure of Choline Therapy to Alter Serum Lipids in Patients with Coronary Artery Disease.* (20548)

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The influence of lipotropic agents upon serum lipids has been studied extensively(1). Chemical studies in patients treated with these drugs over a prolonged period of time have been relatively few; in many of these investigations, the number of determinations have not always been sufficient to minimize the possible error due to spontaneous fluctuations of the serum lipids. For this reason, a long term study was undertaken.

Method. The present observations were made on 11 ambulatory patients, 10 males and one female, ranging in age from 28 to 57 years. Ten subjects had definite arteriosclerosis as evidenced by coronary insufficiency, or previous myocardial infarction, or both; the remaining patient was a member of a family with a striking family history of arteriosclerosis and was suspected of having coronary artery disease. The patients were studied for periods ranging from 6.5 to 15.5 months. Each consumed a self-selected diet. Before starting therapy, at least 3 chemical analyses were done in each case during a control period which varied from one to 5 months. Choline, which is considered the most effective lipotropic agent(2), was given orally in divided doses, as choline dihydrogen citrate (equivalent to 4.5 g of choline base daily), for periods ranging from one to 4 months. These were alternated with control periods ranging from one to 6 months. The serum cholesterol and serum lipid phosphorus were determined at the end of each choline period, at the end of each control period and also at intervals during these periods. The phospholipid:total cholesterol ratio (P/TC) was calculated according to the formula: Lipid P (mg %) x 25/Total Cholesterol (mg %). All determinations were done in duplicate; phospholipids were determined by a modified Whitehorn method(3) and cholesterol by the method of Schoenheimer-Sperry(4). Weight of the patients was recorded at intervals during the study.

Results. Table I summarizes the results obtained. It will be observed that choline therapy did not influence the levels of the total cholesterol and lipid phosphorus when compared to the control values. It is interesting to note that the mean during the control studies for lipid phosphorus was 11.1 mg %, total cholesterol 253 mg % and P/TC 1.10. Under choline therapy, the means for

* Grateful recognition for technical assistance is given to Ann Peranio, and Eileen Flug. Thanks are due to Eli Lilly and Co., Indianapolis, Ind., for supplying the choline dihydrogen citrate.