tween that of rat and guinea pig. For example, rats give 0.25 mg aminopterin daily (11) develop pancytopenia and maturation arrest at level of stem cell within 24 days. Turtles receiving 0.20 mg 5 times weekly develop anemia and leucopenia with enlarged stem cells within 5 to 7 weeks. Guinea pigs receiving 0.25 mg daily develop anemia and leucocytosis after 2 months(12). In this study it has been shown that folic acid is probably necessary in blood formation in the turtle, and in this respect blood formation of reptiles is similar to that of birds and mammals.

Summary. Sex differences in control blood values of box turtles are presented. Mild to toxic doses of cobalt, including mineral supplement and injections of crude liver extract failed to stimulate erythropoiesis. Marked reticulocytosis was induced by repeated bleeding. High reticulocyte counts persisted for 3 weeks following the last bleeding and 4 months were required for restoration of normal erythrocyte values. Blood formation was altered by injections of folic acid antagonist, aminopterin. Arrested maturation of blood cells was detected in form of enlarged marrow blast cells as early as 5 weeks and anemia and leucopenia followed after 6 weeks treatment.

It is a pleasure to thank Mr. Milton Parker for valuable technical assistance.

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Received August 8, 1958. P.S.E.B.M., 1958, v99.

Antimycotic Effect of Spermine.* (24383)

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Spermine, a biologic polyamine, is widely distributed in animal tissues(1) and has also been found in yeasts(2) and bacteriophages (3). Shown to be a growth factor for some micro-organisms(4), it has, on the other hand, been found to inhibit the growth of various bacteria, mainly Gram positive(5). The present communication deals with the effect of spermine on growth of yeasts. Spermidine, a polyamine closely related to spermine was also examined.

Materials. Spermine tetrahydrochloride and spermidine phosphate (Hoffmann La Roche & Co., Ltd., Basle, Switzerland) were used. *Test organisms.* The yeasts examined were obtained from the collection of the Dept. of Bacteriology. *Saccharomyces cerevisiae* served as the main test organism. *Media.* The organisms were grown on the following medium: 0.5% Bacto peptone, 0.5% yeast extract (Difco), 2% glucose, 0.2% $CaCO_3$, 2% agar agar (Difco). The antimycotic effect of spermine was tested in Difco nutrient broth enriched with 0.5% glucose (w/v), pH 7.6.

Antimycotic spectrum of spermine. The antimycotic effect of spermine was tested by the double dilution method(6). Several species

* This investigation was supported by a grant from the Hadassah Medical Organization Research Fund.

TABLE I. Growth Inhibition of Yeasts by Spermine and Spermidine.

Organism		Min conc. of spermine (μg/ml) inhibiting growth after incubation for: 24 hr	1 wk	Min conc. of spermidine (μg/ml) inhibiting growth after incubation for: 24 hr	1 wk
Saccharomyces cerevisiae	22	30	60	>2000	>2000
fragilis	109	"	"	500	"
acidifaciens	16	"	"	"	"
carlsbergensis	7	"	30	"	1000
rosei	20	120	250	1000	>2000
Rhodotorula pallida	127	15	30	30	120
Debaryomyces nicotianae	77	60	60	>2000	>2000
kloeckeri	36	30	"	120	500
Kloeckera apiculata	12	60	120	1000	>2000
Cryptococcus neoformans	10	120	"	"	"
Pichia fermentans	32	"	"	>2000	"
farinosa	23	1000	1000	"	"
Torulopsis sp.	13	250	"	"	"
Oidium sp.	39	500	500	"	"
Candida albicans	26	>2000	>2000	"	"
tropicalis	106	"	"	"	"

Inoculum: 0.1 ml of a 10^{-3} dilution of a 24-hr broth culture.
Temperature of incubation 30°C. Growth inhibition: no visible growth.

of the genus *Saccharomyces, Debaryomyces,* and *Rhodotorula pallida* were the most sensitive (Table I). The human pathogenic *Cryptococcus neoformans* was moderately sensitive to spermine. The growth of most species was not affected by spermidine, although a few were inhibited by high concentrations.

Influence of pH value of the medium and of inorganic salts on the antimycotic action of spermine. Antibacterial activity of spermine has been shown to increase with a rise of pH of the medium(5). A similar effect was observed with *S. cerevisiae.* At pH 4.0 a concentration of 500 μg/ml of spermine was required to inhibit growth of this organism in nutrient broth (Difco), whereas at pH 8.0 a concentration of 30 μg/ml was sufficient. As shown in Table II, inorganic salts interfered with the antimycotic action of spermine. Divalent cations exhibited a higher antagonistic effect than the monovalent ones.

Adsorption of spermine by yeast cells. High quantities of spermine have been shown to be adsorbed by bacterial cells(7). Adsorption of spermine by *S. cerevisiae* and *Candida albicans* was examined as follows: Organisms grown on the solid yeast extract medium for 24 hours at 30°C were harvested and washed 3 times with distilled water. 1.5 ml of cell suspension (containing 50 mg dry weight of cells per ml) were added to 1.5 ml of various concentrations of spermine in 0.05 M boric acid-borax buffer (pH 7.6). After incubation in a shaking bath at 30°C for 1 hour, the cells were sedimented by centrifugation at 7,000 X g for 5 minutes. Amount of spermine in the supernatant was determined chromatographically(8). From the difference between initial

TABLE II. Interference of Inorganic Salts with Antimycotic Action of Spermine.

Salt (M)		Min conc. of spermine (μg/ml) inhibiting growth of *S. cerevisiae* after incubation for: 24 hr	1 wk
NH_4Cl	.03	120	500
	.12	500	1000
	.25	1000	>2000
Na-K-tartarate	.03	250	500
	.12	500	1000
	.25	1000	>2000
KCl	.03	120	1000
	.12	1000	2000
	.25	2000	>2000
$MgCl_2 \cdot 6H_2O$	.03	>2000	"
$MgSO_4 \cdot 7H_2O$	.03	"	"
$CaCl_2 \cdot 2H_2O$	.03	"	"
Control, without salt		60	60

Medium: Difco nutrient broth enriched with 0.5% glucose and one of the inorganic salts indicated. Temperature of incubation 30°C. Growth inhibition: no visible growth.

TABLE III. The Amount of Spermine Adsorbed by Cells of *S. cerevisiae* and *C. albicans* at Various Concentrations of Spermine in the Medium.

Conc. of spermine in buffer (μg/ml)	*S. cerevisiae*		*C. albicans*		*S. aureus*	
	Spermine adsorbed	Spermine eluted from cells	Spermine adsorbed	Spermine eluted from cells	Spermine adsorbed	Spermine eluted from cells
	(μg/mg dry wt of cells)					
200	2.0	.7	1.7	.5	8.0	3.5
400	2.8	1.1	2.5	.6	18.0	7.0
600	4.0	1.3	3.5	.9	22.0	8.5
1000	8.0	3.3	6.0	1.1	22.0	10.0

concentration of spermine and concentration of spermine in the supernatant the amount of the drug bound by the cells could be determined. The amount of spermine adsorbed by the yeast cells rose with its concentration in the buffer (Table III). There was no significant difference between the amount adsorbed by the spermine sensitive *S. cerevisiae* and the spermine resistant *C. albicans*. The amount of spermine adsorbed by *Staph. aureus* was found to be considerably higher (Table III). Part of the spermine adsorbed by the cells could be eluted by mineral acids. The cells, after adsorbing spermine, were washed once with boric acid-borax buffer (pH 7.6) and agitated for 1 hour in 0.1 N HCl at room temperature. The amount of spermine eluted was determined chromatographically (Table III). Twenty to 40% of the adsorbed spermine could be eluted from the cells. The percentage of the eluted substance was somewhat higher with *S. cerevisiae* than with *C. albicans*.

Agglutination of yeast cells by spermine. Spermine has been shown to agglutinate various bacteria(7). In order to determine whether yeast cells can be agglutinated by spermine the following experiment was performed. Serial double dilutions of spermine in distilled water were set up, each tube receiving 2 ml of solution and thereafter 0.2 ml of distilled water containing 2 x 10^7 washed cells. The tubes were incubated for 2 hours at 37°C and inspected for agglutination; they were then left overnight at 6°C and reinspected. When agglutinated the yeasts sedimented adhering firmly to the bottom of the tube, forming a circle with an irregular border. When not agglutinated a circular button-like sediment developed. *S. cerevisiae* and *C. albicans* were agglutinated by spermine in a concentration as low as 3 μg/ml.

Effect of spermine on washed cells of S. cerevisiae. The antibacterial effect of spermine has been shown to depend on cellular metabolic activity(7). To see whether spermine will exert its effect only on metabolizing yeast cells the following experiment was carried out. Yeast cells grown on Difco nutrient broth enriched with 0.5% glucose for 24 hours at 30°C were harvested, washed 3 times with distilled water and resuspended in the same volume of distilled water. 1 ml of a 10^{-2} dilution of the suspension was inoculated into 9 ml of tris buffer containing 200 μg/ml or 400 μg/ml of spermine and also into tris buffer with the same spermine concentrations enriched with 0.4% glucose. The same media without spermine served as controls. As may be seen from Fig. 1, cells died out more rapidly in tris buffer enriched with glucose than in tris buffer alone. When spermine was added to the buffer the rate of death slowed down; 400 μg/ml of spermine showed a higher protective effect than 200 μg/ml. The same amounts of spermine added to tris buffer en-

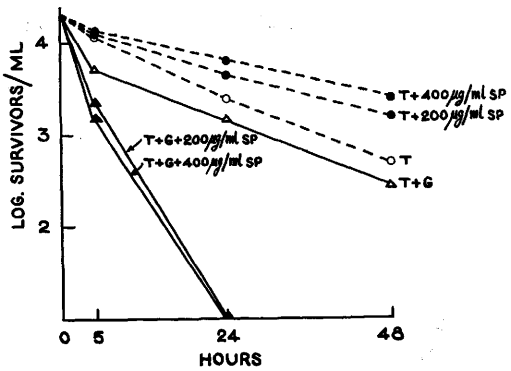

FIG. 1. Effect of spermine on washed cells of *S. cerevisiae* suspended in buffer in presence or absence of glucose. T—0.05 M tris-(hydroxymethyl)-aminomethane buffer, pH 7.6; G—0.4% glucose (w/v); SP.—spermine tetrahydrochloride.

riched with glucose exhibited a pronounced killing effect.

Comment. Spermine is present in rather high concentrations in some human tissues and might play a role as a natural antibacterial substance(8,9). Our findings suggest that spermine might influence *Cryptococcus* infections to a certain degree, but not infections due to *Candida*. *Candida* species were unable to oxidise spermine (unpublished data), hence the high spermine resistance shown by these yeasts cannot be explained by the presence of a spermine oxidase. It is of interest that the highly spermine sensitive *S. cerevisiae* contains appreciable amounts of spermine and spermidine(2). Yeasts adsorbed a smaller amount of spermine than bacteria(7). This phenomenon might be explained by the smaller surface: volume quotient of the yeast cell when compared with that of the bacterial cell. The mechanism of the antimycotic effect of spermine seems to be similar to the antibacterial one. The same factors influence antibacterial and antimycotic action: *i.e.* pH of the medium, presence of inorganic salts, and metabolic activity of the cell.

Summary. 1) Spermine inhibited growth of several species of yeasts, *Saccharomyces* and *Debaryomyces* species being the most sensitive. The antimycotic effect of spermine was more pronounced at an alkaline pH and was antagonized by inorganic salts. The adsorption of spermine by yeast cells was significantly smaller than by *Staph. aureus*. 2) Spermine retarded the death rate of washed cells of *S. cerevisiae* suspended in tris buffer. A pronounced killing effect of spermine was observed when glucose was added to the buffer.

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Received August 25, 1958. P.S.E.B.M., 1958, v99.

Effect of Δ-4-Cholestenone on Cholesterol and Phospholipid Metabolism in the Chick. (24384)

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There is increasing interest in the hypothesis that atherosclerosis might be successfully treated by use of materials that inhibit endogenous cholesterol synthesis. One such compound, Δ-4-cholestenone, was first shown by Tompkins *et al.*(1) to diminish the incorporation of labeled acetate into the cholesterol of liver slices taken from rats treated with the material. More recently these investigators (2) reported that *in vivo* cholestenone depressed plasma cholesterol levels in normal dogs and chickens. In addition, Steinberg and Fredrickson(3) demonstrated that when rats were treated with this compound there was a decrease in serum cholesterol and a diminution of incorporation of labeled acetate into hepatic cholesterol. Both groups of workers have emphasized that this compound has serious undesirable side effects, *e.g.*, Chaikoff and colleagues have shown it to be a precursor of cholestan-3β-ol(4) an atherogenic material in both rabbits and chickens(5,6). Furthermore, Steinberg *et al.*(7) have very recently indicated that while cholestenone may have been ineffective in modifying serum cholesterol levels in 2 hypercholesterolemic patients, their