when 2.5 mg of reserpine is given intravenously to dogs. This may be an additional mechanism for the increased incidence of ulceration.

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Use of Fungizone® in Control of Fungi and Yeasts in Tissue Culture. (26509)

D. PERLMAN, NANCY A. GIUFFRE AND SHARON A. BRINDLE
(Introduced by R. Donovick)
Squibb Institute for Medical Research, New Brunswick, N. J.

Fungal and yeast contamination is a troublesome problem to those working in tissue culture laboratories. Mycostatin (Squibb Nystatin), an antifungal antibiotic derived from *Streptomyces noursei* was found by McLimans *et al.*(1) to have little or no effect on growth of mammalian cells and to suppress growth of fungi and yeasts effectively. As it is relatively insoluble in aqueous media, it has been difficult to use in certain tissue culture systems, especially where the cells are being grown in monolayers. Fungizone®*, the sodium deoxycholate complex of amphotericin B(2) an antifungal agent produced by *Streptomyces nodosus*(3), forms a colloidal dispersion in aqueous media and is effective in suppressing growth of many yeasts and fungi(4). Hemphill *et al.*(5) found that Fungizone was effective in controlling growth of certain yeasts and fungi in tissue cultures used for propagation of viruses, and observed no deleterious effects of Fungizone on viral multiplication in the infected cells or of cell multiplication of the uninfected cells. We have studied the effect of Fungizone on growth of a number of established cell lines and of chick fibroblasts in monolayer culture, and have confirmed that Fungizone when used in certain fungistatic concentrations, does not noticeably affect cell multiplication. We have also studied the stability of Fungizone in a number of tissue culture media and determined rate of inactivation at 37°C in these menstra.

Methods. Four media were used in these experiments including Eagle's medium(6) with 10% (v/v) calf serum, Ziegler's modification (7) of Eagle's medium containing 10% (v/v) calf serum, Waymouth's chemically defined medium MB 752/1(8) supplemented with 10% (v/v) calf serum and 0.03% carboxymethylcellulose and Waymouth's medium supplemented with 0.5% carboxymethylcellulose. Among the cell lines used in these studies were Earle's L_{929} strain of mouse fibroblasts, the HeLa (Gey) cell line, a cell line derived from bovine pituitaries, and 2 cell lines derived from samples of peritoneal fluid from mice infected with Ehrlich ascites tumors. Chick fibroblasts were prepared by

* Fungizone is the registered trade mark of E. R. Squibb and Sons for the amphotericin B sodium deoxycholate complex.

trypsinization of minced tissue and grown in Eagle's medium in monolayers.

Milk dilution bottles (40cm^2 surface) and Falcon plastic flasks (25 cm^2 surface) were used for the monolayer cultures, while suspension cultures were grown in 1 $\times$ 6 inch screw capped tubes placed on the Rollordrum apparatus as described earlier(9). Cell counts were determined with a Coulter Counter and ID_{50} values determined graphically from dose response curves. Inoculum for the suspension cultures was adjusted to 100,000 cells per ml and after 3 to 5 days' incubation the populations in the control tubes had increased to between 450,000 and 750,000 cells per ml depending on the cell line. Smaller inocula were used for the cultures grown in monolayers, and microscopic observations were made at 2 day intervals to determine the effect of Fungizone on cell morphology and multiplication.

Fungizone concentrations of supplemented media were determined by a bioassay using *Saccharomyces mellis* as test organism(10).

Results. A study of the stability of Fungizone in tissue culture media incubated up to 7 days at 37°C is summarized in Table I. The presence of serum in the media apparently stabilized the antifungal agent, and the presence of growing cells had no effect on the stability of Fungizone in these systems. As most cultures of yeasts and fungi are completely inhibited by 1 μg of Fungizone per ml, there still would be sufficient antifungal activity left after 7 days' incubation of media initially containing 5 μg Fungizone per ml, and a great excess when the initial concentration was 50 μg per ml. No loss of antifungal potency was found when these media supplemented with 5 or 50 μg per ml of Fungizone were stored at 4°C for 7 days, and no precipitation of the antifungal agent from the medium was observed.

The effectiveness of Fungizone in inhibiting growth of yeasts and fungi added to Eagle's tissue culture medium was also examined. Among the cultures used in these experiments were *Aspergillus niger, Penicillium notatum,* unidentified *Penicillium* and yeast cultures isolated from contaminated tissue culture, *Rhizopus arrhizus, Saccharomyces cerevisiae* and *Candida albicans.* A concentration of 0.5 μg of Fungizone per ml was effective in inhibiting the growth of the fungi and yeasts when initial inocula were 10,000 and 100,000 mold spores per ml, and 100,000 and 1 million yeast cells per ml. Cultures were incubated at 37°C for 4 to 7 days. These results confirm and extend the observations reported by Hemphill *et al.*(5).

Observations on the sensitivity of the cell lines to Fungizone are summarized in Table II. Most of the cultures when grown in ser-

TABLE I. Stability of Fungizone in Tissue Culture Media Incubated at 37°C.

	% of antifungal activity* remaining after incubation for stated period at 37°C							
	Initial conc. 5 μg/ml				Initial conc. 50 μg/ml			
	Medium without cells		Medium with cells†		Medium without cells		Medium with cells†	
Medium	4 days	7 days	4 days	7 days	4 days	7 days	4 days	7 days
Eagle's medium containing 10% (v/v) calf serum	39	20	57	42	78	67	80	76
Ziegler's modification of Eagle's medium with 10% (v/v) calf serum	56	40	40	32	74	67	78	71
Waymouth's medium MB 752/1 supplemented with 10% (v/v) calf serum	54	34	65	38	58	58	33	25
Waymouth's medium MB 752/1 supplemented with 0.5% carboxymethylcellulose	13	11	18	18	73	54	66	57

* As determined by bioassay using *Saccharomyces mellis* as test organism.
† Medium inoculated with Earle's L_{929} culture of mouse fibroblasts.

TABLE II. Effect of Fungizone® on Multiplication of Cells in Tissue Culture.

Cell line	Method of growth	Medium	ID_{50}, μg/ml	Other observations
L_{929} (mouse fibroblast)	Suspension	A	25	
	"	B	2.5	
	"	D	30	
	Monolayer	A		No inhibition at 25 μg/ml
HeLa	Monolayer	A		*Idem*
Bovine pituitary	Suspension	A	25	
Ehrlich ascites (R)	"	A	35	Slight inhibition at 5 μg/ml
" " (S)	"	A	35	*Idem*
Chick fibroblast	Monolayer	C		No inhibition at 25 μg/ml

Composition of media: A—Waymouth's MB 752/1 supplemented with 10% (v/v) calf serum.
B—Waymouth's MB 752/1 supplemented with 0.3% carboxymethylcellulose.
C—Eagle's medium containing 10% (v/v) calf serum.
D—Ziegler's modification of Eagle's medium with 10% (v/v) calf serum.

um-containing media were not inhibited by addition to the media of 2.5 μg to 5 μg of Fungizone per ml. Some of the cytotoxicity of Fungizone is perhaps due to the deoxycholic acid component of the preparation as growth of most of the cell lines tested was found to be at least slightly inhibited by 25 μg per ml(11). Amphotericin B (dissolved in dimethylformamide) caused 50% inhibition of growth of L cells in suspension when added to the medium at a concentration of 50 μg per ml. This inhibition was associated with the amphotericin B as addition of an equivalent amount of dimethylformamide (0.004 ml per ml of medium) did not affect cell growth.

In experiments studying the effectiveness of Fungizone in eliminating growth of yeasts from tissue culture, approximately 1,000 *S. cerevisiae* cells were added to a 25 ml suspension culture of L cells contained in 1 × 6 inch screw-capped test tubes. After 16 hours incubation on the Rollordrum apparatus the yeast cell count had reached about 1,000,000 cells per ml. Fungizone was then added to several of the tubes to give concentrations ranging from 2.5 to 25 μg per ml and the tubes replaced on the Rollordrum apparatus. After 24 hours incubation the yeast count in all the tubes had dropped noticeably. The tubes were centrifuged, the collected cells resuspended in media containing 2.5 μg of Fungizone per ml, and the tubes replaced on the Rollordrum apparatus. After 48 hours incubation the L cell count had doubled and no evidence was seen of viable yeast on microscopic inspection. The L cells were again collected by centrifugation, and resuspended in media without Fungizone or other antifungal agents. No evidence of yeast cell growth was found after 3 days' incubation, and the L cells appeared "normal" when examined microscopically. In another study, a penicillium culture was found growing in a suspension culture of a cell line taken from bovine pituitary tissue. Addition of Fungizone to give a concentration of 2.5 μg/ml resulted in inhibition of growth of the penicillium, and after 2 subcultures (at 3 day intervals) the cell line was found to be free of the contaminant. Fungizone at 2 μg/ml was also effective in inhibiting growth of another penicillium contaminant found in a monolayer culture of a cell line derived from Ehrlich ascites cells. Since 0.5 μg of Fungizone per ml of medium is sufficient to control fungal and yeast contamination, we conclude that the use of 2 μg to 5 μg per ml of medium may be used to eliminate fungal and yeast contaminants from tissue cultures without significantly damaging the tissue cultures. Continued use of media supplemented with serum and 10 μg of Fungizone per ml of medium for growth of Earle's L_{929} cell line in suspension cultures did not result in any change in cell multiplication rates during the 3 week observation period.

Summary. In experiments studying the effect of Fungizone (the sodium deoxycholate

complex of amphotericin B) on growth of established cell lines and chick fibroblasts in monolayer and suspension culture systems, the fungistatic concentration of 2.5 μg/ml was found to have little or no effect on the multiplication of the tissue cultures grown in serum-containing media. This concentration was tolerated by all cultures. Inactivation of Fungizone in tissue culture media was also determined.

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Carbohydrate Content of Euglobulins of Normal and Rheumatoid Sera. (26510)

WILLARD R. STARNES (Introduced by Emmett B. Carmichael)
Metabolic Research Laboratories, Veterans Administration Hospital, Birmingham, Ala.

The term "rheumatoid factor" has been applied to the protein component of serum which is responsible for a group of agglutination and precipitation reactions, some of which are very important in diagnosis of rheumatoid arthritis. Up to the present, analytical studies have indicated that the rheumatoid factor is a typical 19-S gamma globulin with chemical, physical and immunological properties closely akin to 19-S antibodies(1). The present studies were undertaken to isolate euglobulins by different methods and study the carbohydrate composition. Franklin, Muller-Eberhard and Kunkel isolated and characterized, by various methods, a refined and more purified "rheumatoid factor" preparation upon which they determined the various carbohydrate fractions(1). Others have also achieved considerable purification by other technics(2,3).

Materials and methods. Known normal pooled sera and pooled sera with a positive latex agglutination were submitted to 3 different methods for isolation and purification of the euglobulin fractions. (1) *Precipitation with 0.0027 N hydrochloric acid:* Test sera were inactivated by heating at 56°C for one-half hour. In a 100 ml cellulose nitrate tube, one volume of clear serum was diluted by the slow addition (with continuous rotation of the tube) of 9 volumes of 0.0027 N hydrochloric acid from a 50 ml burette. A pH within the range of 6.1 to 6.4 was thereby attained. The tubes were immediately placed in a refrigerated centrifuge at 4 to 8°C for 30 to 60 minutes. The euglobulin precipitate then was centrifuged in the cold at 3,000 rpm for 10 minutes. The clear supernate was discarded by decanting from the firmly packed pellet, and last traces of the supernate were removed either by draining the tube inverted over filter paper or by wiping the inner wall thoroughly with a gauze sponge. The precipitate was not washed. The concentrated euglobulin was then dissolved in an added 0.5 ml of 0.9% NaCl solution, (2) Svartz cold agglutination, (3) and isolation and purification by continuous electrophoresis in veronal buffer 0.05 M pH 8.6. The euglobulin was subjected to 3 separations on