now in progress to quantitate this serological test.

Summary. By immunizing rabbits with a lipoprotein fraction obtained from the serum of a dog on a cholesterol-thiouracil atherogenic diet, it has been possible to produce immune sera which will detect the abnormally increased concentration of serum lipoproteins in dogs on cholesterol-thiouracil atherogenic diets and which will react with the serum from an atherosclerotic man. Sera of normal dogs tested with this immune serum by the precipitin technic, consistently show a lipoprotein concentration significantly less than that observed in the case of dogs on the atherogenic dietary regimen. Sera from three men in whom no vascular disease had been detected, did not react with the immune rabbit sera.

1. Baker, S. P., Ogden, E., and Riddle, J. W., *Texas Reports on Biol. and Med.,* 1951, v9, 391.

2. Gofman, J. W., Lindgren, F. T., Elliott, H., Mantz, W., Hewitt, J., Strisower, B., and Herring, V., *Science,* 1950, v111, 166.

3. Baker, S. P., Leet, R. H., Ogden, E., and Van Winkle, Q., Abstract in *Am. J. Physiol.,* Dec., 1952, Presented at Sept. 1952 Meeting, Am. Physiol. Soc., New Orleans, La.

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In vitro Studies with 1-hydroxy-2(1H)pyridinethione. (20041)

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Shaw *et al.*(1) in their work on analogs of aspergillic acid reported the preparation and properties of 1-hydroxy-2(1H)pyridinethione, the sodium salt of which is designated MC 3277.* Because of its striking activity against a very wide group of microorganisms further studies were undertaken. The present report will discuss certain antibacterial and antifungal characteristics of this compound.

Assay of MC 3277. A turbidimetric tube assay has been developed using as an assay organism, *Saccharomyces cerevisiae* Squibb strain, and Difco penassay broth modified to contain 1% dextrose concentration as a test medium. Otherwise the assay procedure of Donovick *et al.*(3) is used unchanged. Statistical analysis† of data obtained with the assay shows that for a single assay on any one day, the assay will be within 8.5% of the true value 95% of the time.

In vitro spectrum. A. *Antibacterial.* The sensitivities of a group of microorganisms to MC 3277 were determined as follows: A 2-fold dilution procedure was used with incubation for 17 hours at 37°C for most organisms. Unless otherwise stated, Difco penassay broth was used as a test medium. Cultures in the logarithmic phase of growth with approximately 1,000 organisms per ml were used where possible. The amount of MC 3277 required to inhibit completely the growth of the organism as measured by an absence of turbidity is reported as the Minimal Inhibiting Concentration (M.I.C.). In Table I are listed the M.I.C.'s for a representative group of microorganisms. The M.I.C.'s range from 0.006 μg/ml for *M. tuberculosis* var. *bovis* Strain BCG to 4.0 μg/ml for *Pseudomonas aeruginosa.* The gram-positive organisms are most sensitive but the range of activity for the gram-negative group, from 1.5 μg/ml for *Klebsiella pneumoniae* and *Salmonella typhosa* to 4.0 μg/ml for *Ps. aeruginosa* is of a high order.

B. *Antifungal.* The action of MC 3277 against a group of molds, yeasts and yeast-like fungi was determined by the streak-plate method using a malt-yeast extracts dextrose agar. In Table II are reported the M.I.C.'s found for these fungi. The activity

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TABLE I. Antibacterial Spectrum of MC 3277.

Organism	M.I.C. in μg/ml	Medium
Micrococcus pyogenes var. *aureus* 209P	.13	Difco penassay broth
Klebsiella pneumoniae	1.5	"
Pseudomonas aeruginosa	4	"
Streptococcus faecalis	.5	"
Shigella dysenteriae	.5	"
Streptococcus pyogenes C-203	.25	Difco brain heart infusion
Salmonella schottmülleri	2.5	Difco penassay broth
Aerobacter aerogenes	3.5	"
Escherichia coli	2.5	"
Proteus vulgaris	.7	"
Mycobacterium tuberculosis var. *bovis* strain BCG	.006	Modified Kirchner's*
Salmonella typhosa	1.5	Difco penassay broth
Lactobacillus acidophilus	.4	"
Clostridium septicum	.4	Fluid thioglycollate medium
Brucella abortus	.4	Tryptose semi-solid
Neisseria catarrhalis	.6	Difco brain heart infusion
Corynebacterium pyogenes	.3	Squibb diphtheria medium
Streptococcus agalactiae	.08	Difco penassay broth
Bacillus subtilis	.06	"

* Donovick, R., Pansy, F., Stryker, G., and Bernstein, J., *J. Bact.*, 1950, v59, 667.

TABLE II. Antifungal Spectrum of MC 3277.

		M.I.C. in μg/ml of agar
Aspergillus niger		.6
A. fumigatus		.3
Penicillium notatum		.3
Ceratostomella ulmi		.08
Trichophyton mentagrophytes #1920		1
T. mentagrophytes Squibb		.15
T. tonsurans		1
T. rubrum		1
T. schoenleini		.5
T. violaceum		2
T. faviforme var. *discoides*		.03
T. faviforme var. *ochraceum*		.03
Microsporum audouini		.3
M. audouini (Kligman strain)		<.06
M. canis		1
Epidermophyton flocossum		.25
Sacch. cerevisiae		.08
S. pastorianus		.04
Candida albicans		.08
Cryptococcus neoformans	(in Difco Penassay broth + .5% glucose)	.10

of the pyridinethiones against the fungi is particularly striking. The most resistant organism found was *Trichophyton violaceum* which was inhibited by 2 μg/ml. Most of the other fungi are much more sensitive. Of particular interest are the very high activities against *Candida albicans* and *Cryptococcus neoformans.*

Action of various body fluids on the action of MC 3277. Since, in preliminary tests vs. *Streptococcus pyogenes* C-203, MC 3277 showed no activity in mice, it was of interest to determine whether this compound was easily inactivated by body fluids. The action of saliva, cerebrospinal fluid and serum on MC 3277 was accordingly determined. More extensive inactivation studies are reported by Thomas *et al.*(2).

A. Saliva. Four organisms, *M. pyogenes,* var. *aureus, Streptococcus pyogenes, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa,* were tested in a basal medium (Difco penassay broth) and in the same medium with 30% of the water substituted by saliva. The media were sterilized by Seitz filtration and M.I.C.'s of 1-hydroxy-2(1H)pyridinethione were determined by a 2-fold procedure in the two media. The results, reported in Table III, show some degree of inactivation for *Strep. pyogenes, K. pneumoniae,* and *Ps. aeruginosa,* and a high degree of inactivation for *M. pyogenes* var. *aureus.* In a similar manner MC 3277 was tested against *Saccharomyces cerevisiae* in the presence and absence of 0.1% mucin. An inactivation of MC 3277 of about 25% was observed.

B. *Cerebrospinal fluid.* Media were prepared as in A above using 30% human cerebrospinal fluid (CSF) as the water substitute. The M.I.C.'s of MC 3277 were obtained against *M. pyogenes* var. *aureus* and *Crypto-*

TABLE III. Action of Saliva on Activity of 1-hydroxy-2(1H)pyridinethione.

Organism	M.I.C. in μg/ml: In basal medium	M.I.C. in μg/ml: Basal medium + 30% saliva
M. pyogenes var. *aureus* 209P	.15	3.5
Strep. pyogenes C-203	.3	.6
K. pneumoniae	2	3
Ps. aeruginosa	5	6

TABLE IV.
Effect of Serum on Activity of Several Concentrations of MC 3277 against *Saccharomyces cerevisiae.*

Concentration, μg/ml	M.I.C. in μg/ml at 0 hr	1 hr	3 hr	24 hr
A) 50% Bacto beef serum at 37°C				
1000	.0261	.0230	.0245	.0285
500	.0277	.0238	.0238	.0245
100	.0230	.0223	.0277	.0277
5	.0328	.0384	.0396	.0592
B) Distilled water at 37°C				
1000	.0277	.0238	.0253	.0245
500	.0253	.0260	.0268	.0317
100	.0245	.0238	.0245	.0223
5	.0306	.0317	.0357	.0317

coccus neoformans in both media. The M.I.C. of 0.15 μg/ml for *M. pyogenes* var. *aureus* and 0.10 μg/ml for *C. neoformans* was unchanged by the presence of CSF after 2 days incubation at 37°C.

C. *Serum.* Weighings of MC 3277 were made up in 50% sterile Bacto beef serum and sterile distilled water. The levels made up were 1,000 μg/ml, 500 μg/ml, 100 μg/ml, and 5 μg/ml. Aliquot portions were drawn off and assayed immediately and the samples then placed at 37°C. At one hour, 3 hours, and 24 hours, portions were drawn off and assayed by the method described above. The data (Table IV) indicate that at concentrations of 1,000 μg/ml, 500 μg/ml, and 100 μg/ml, there is no apparent inactivation. At 5 μg/ml in 50% Bacto beef serum, however, there appears to be some inactivation after 24 hours.

Summary and conclusions. An antibacterial and antifungal spectrum for the sodium salt of 1-hydroxy-2(1H)pyridinethione as well as an assay method for the same is presented. *In vitro* the substance is partially inactivated by saliva and mucin, by serum at low concentration, but not by human cerebrospinal fluid. The inactivation by saliva, mucin, and serum however may be of relatively minor importance in view of the extraordinarily high activity displayed by this compound.

1. Shaw, E., Bernstein, J., Losee, K., and Lott, W. A., *J. Am. Chem. Soc.*, 1950, v72, 4362.
2. Thomas, B. G. H., *et al.*, to be published.
3. Donovick, R., Hamre, D., Kavanagh, F., and Rake, G., *J. Bact.*, 1945, v50, 623.

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Antibiotic Combinations and Resistance to Antibiotics: Penicillin-Erythromycin and Streptomycin-Erythromycin Combinations *in vitro*.* (20042)

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The emergence of penicillin-resistant strains of staphylococci during prolonged exposure to that antibiotic *in vitro* or in the course of therapy is now a well established phenomenon. In many large hospitals where penicillin is used very extensively this phenomenon is probably responsible for the complete reversal from predominantly sensitive to predominantly resistant strains of staphylococci among those isolated from infected materials and carriers in the hospital populations(1-6). It has recently been demonstrated that the new antibiotic erythromycin is highly and equally active against both penicillin-sensitive

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