TABLE I.
Comparative Findings by Other Grouping Technics on 791 Strains Successfully Grouped by the Maxted Technic.

Classification by the enzyme method Group	Method or methods used for comparison								
	Formamide and hot acid			Formamide			Hot acid		
	No. strains	Same group	Neg. reaction	No. strains	Same group	Neg. reaction	No. strains	Same group	Neg. reaction
A	35	35	0	287	287	0	249	249*	0
B	0	0	0	0	0	0	45	35	10†
C	1	1	0	3	3	0	82	69	13†
D	0	0	0	0	0	0	3	0	3†
F	0	0	0	0	0	0	3	3	0
G	0	0	0	4	4	0	79	59	20†
Total	36	36	0	294	294	0	461	415	46†

* In approximately 18% of these group A strains it was necessary to repeat the test before a precipitin reaction was obtained.

† These non-group A strains were lost before a second hot acid extract could be prepared.

together satisfactory. In many instances the precipitin reaction obtained was minimal and transient. A stronger reaction could be obtained by diluting the extract, by using different lots of antisera or by preparing a new extract. All strains which were so checked eventually gave a positive result with the hot acid extract which confirmed that obtained with the Maxted extract. Using the hot acid technic, it was necessary to repeat the tests on approximately 18% of the strains of streptococci as compared to approximately 2% with the formamide technique and less than 1% with Maxted's technique.

Stronger precipitin reactions are usually obtained with the Maxted extracts than with the formamide and hot acid extracts, thus simplifying the reading of the tests. The reactions employing the Maxted extracts should be read after one hour of incubation since cross reactions may develop after overnight refrigeration.

Of practical importance is the fact that group classification by Maxted's technique can be completed within a few hours after the streptococci have been isolated in pure culture on a blood agar plate. Also, the only procedure required for making the extracts is a transfer of the organisms into the *Streptomyces* preparation.

Summary. Experience with 1010 consecutive strains of hemolytic streptococci examined by Maxted's grouping technique is presented. Classification by this method was successful with 998 strains. Comparison with the older formamide and hot acid techniques suggests that Maxted's method is reliable and is simpler to perform.

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Use of Mucin in Experimental Infections of Mice with *Histoplasma capsulatum*. (17716)

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The use of mucin as a resistance-lowering substance for bacterial and virus infections recently has been reviewed in detail by Olitzki (1). No published data have been cited, however, to suggest that mucin has ever been employed as an adjuvant in establishing experi-

1. Olitzki, L., *Bact. Rev.*, 1948, v12, 149.

TABLE I.
Effect of Mucin on Virulence of *H. capsulatum* for Mice.

Strain No.	Group	Challenge dose of *H. capsulatum* Million	No. days after inj.	No. deaths in mice inoculated with organisms suspended in: Saline	5% mucin
G-2	A	35	9	0/10*	10/10
	B	7	20	0/10	10/10
	C	0.7	30	0/10	3/10
G-5	A	35	30	0/10	8/10
	B	7	30	0/10	4/10
	C	0.7	30	0/10	1/10
G-6	A	35	30	0/10	5/10
	B	7	30	0/10	1/10
	C	0.7	30	0/10	0/10
G-8	A	35	11	0/10	10/10
	B	7	17	0/10	10/10
	C	0.7	30	0/10	4/10
G-8 control	†	7	30	—	0/20
Mucin control	‡	—	30	—	0/20

* In this and other tables the numerator represents the number of deaths and the denominator the total number of mice in the experimental groups.
† Organisms were autoclaved before suspending in mucin.
‡ 1 ml of 5% mucin was administered by the intraperitoneal route.

mental infections with the pathogenic fungi. The evaluation of certain chemotherapeutic agents in *Histoplasma capsulatum* infections has been restricted by the relative resistance of white mice to this organism administered by the intraperitoneal route. In order to study the action of chemotherapeutic agents in the treatment of experimental histoplasmosis, it was therefore first necessary to devise an infective technic that would insure consistently high rates of infection and mortality in untreated mice. The successful employment of mucin as an enhancing agent in certain bacterial and virus infections suggested the desirability of investigating its capacity to "enhance" the virulence of *H. capsulatum* for mice.

Methods. Inocula were prepared from yeast phase cultures of *H. capsulatum* grown on glucose cystine blood agar(2) for 4 days at 37°C. The growth was washed from the slants and diluted with sterile normal salt solution to yield a density of 0.500 (±.010) at 650 γ on the Coleman Junior Spectrophotometer. This suspension, from which all subsequent dilutions were prepared contained approximately 70 million organisms per ml by direct haemocytometer count. Cultural characteristics of the organism in this growth phase made it impracticable to attempt to determine the actual number of viable cells by plate count. The 5% gastric mucin employed was prepared by the Department of Biologic Products of this institution according to the method they routinely use, which is as follows: Granular hog mucin (Wilson, Type 1701-W) is suspended to 5% concentration in distilled water with the aid of a Waring blendor. The suspended material is kept in the refrigerator for 36 hours with occasional shaking, then warmed and adjusted to pH 7.2, filtered through gauze and cotton, and sterilized by heating for 30 minutes in a boiling water bath 3 times within a period of 24 hours. Tests for sterility, toxicity and "virulence-enhancing" activity (in *Salmonella typhosa* infections of mice) are routinely performed. White Swiss mice (Bagg strain) weighing 14-17 g were used throughout these studies and were distributed in groups of 5 mice each in individual jars by a random

2. Campbell, Charlotte C., *J. Bact.*, 1947, v54, 263.

TABLE II.
Summary of Results of 5 Experiments. Mortalities in Mice Infected with the G-8 Strain of *H. capsulatum* Suspended in Mucin.*

No. days after injection	Groups											
	A		B		C		D		E		Total	
	No.	%†	No.	%	No.	%	No.	%	No.	%	No.	%
7	0/40	0	0/37	0	0/39	0	1/40	2.5	0/47	0	1/203	0.49
14	22/40	55	18/37	48.6	17/39	43.6	19/40	47.5	25/47	53.3	101/203	49.7
21	30/40	75	25/37	67.5	22/39	56.4	31/40	77.5	31/47	66	139/203	68.4
30	34/40	85	28/37	75.7	26/39	66.6	31/40	77.5	39/47	83	158/203	77.8

See Table I for legend.
* Inoculum contained approximately 3.5 million organisms.
† % deaths.

procedure.

Results. The results obtained with saline and mucin suspensions of 4 yeast phase strains of *H. capsulatum* (G-2, G-5, G-6 and G-8)* are compared in Table I. Challenge doses were administered to 4 groups of 60 mice each. Ten mice in each group received comparable volumes of the organism suspension in 5% mucin, and 10 mice equal dilutions in saline, representing 35 million, 7 million and 700,000 organisms, respectively. Challenge doses of the G-2 strain of *H. capsulatum* suspended in 5% mucin killed all mice in groups A and B (Table I), while in group C only 3 of 10 mice died during the 30 day period. The mortality pattern obtained with the mucin suspension of strain G-8 was similar to that of G-2, but strains G-5 and G-6 administered in mucin were less effective. While it is evident that the "virulence-enhancement" activity of mucin suspensions varied with different strains of the organism it should be noted that no deaths occurred among animals receiving equivalent challenge doses suspended in saline.

To exclude the possibility that neither the mucin nor the mucin-organism suspension was inherently toxic, 2 control groups of 20 mice each were included (Table I). The mice in one group were each injected with 7.0 million G-8 organisms which had been killed by autoclaving before suspending in mucin. Mice in the second control group were inoculated with 1 ml of the 5% mucin suspension which was to be employed as diluent for inocula in this and subsequent experiments reported in this paper. All mice in both control groups survived the 30-day observation period.

Since human histoplasmosis frequently displays a clinical picture of chronicity it was believed that more effective evaluation of chemotherapeutic agents could be obtained if a challenge dose were established which would consistently produce mortality in excess of 50% only after a 2 to 3 week period following injection. A challenge inoculum that would serve this purpose was derived from additional pilot studies with strain G-8. This inoculum consisted of 0.5 ml of a 1:10 dilution of the organism suspension in mucin (approximately 3.5 million organisms). The results of 5 experiments performed at monthly intervals in which this challenge dose was employed are shown in Table II. A total of 210 mice, divided into 4 groups of 40 mice each and 1 group of 50, were inoculated and observed for periods of 30 days. Occasional deaths occurring within the first 3 days after injection were believed not to have been the result of *H. capsulatum* infection and were not included in the final calculations. Mortalities in the various groups varied from 0 to 2.5% by the 7th day, from 43.6 to 55% by the 14th day, from 56.4 to 77.5% by the 21st day and from 66.6 to 85.0% by the 30th day. The average death rates were 0.49, 49.7, 68.4 and 77.8% at 7, 14, 21 and 30 days, respectively.

The marked difference between mucin and saline suspensions observed in the early ex-

* The G-2, G-5 and G-6 strains of *H. capsulatum* are from the collection of Dr. N. F. Conant, Duke University. The G-8 strain, a human isolate, was identified at this institution.

TABLE III.
Comparison of Mortalities in Mice Receiving Equivalent Challenge Doses of *H. capsulatum* (G-8) Suspended in Mucin and Saline.*

No. days after injection	Groups F				G				H				Total			
	Saline		Mucin		Saline		Mucin		Saline		Mucin		Saline		Mucin	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
14	0/40	0	19/40	47.5	1/47	2.1	25/47	53.3	0/20	0	10/20	50	1/107	0.9	54/107	50.5
21	1/40	2.5	31/40	77.5	2/47	4.3	31/47	66	0/20	0	13/20	65	3/107	2.8	75/107	70.1
30	1/40	2.5	31/40	77.5	2/47	4.3	39/47	83	0/20	0	15/20	75	3/107	2.8	85/107	79.4

See Table I for legend.
* Inoculum consisted of approximately 3.5 million organisms.

periments was further substantiated with 3 additional groups of mice. These groups consisting of 40, 50 and 20 mice, respectively, were inoculated with 0.5 ml of saline suspension containing approximately 3.5 million organisms. Inoculations for groups F and G (Table III) were performed simultaneously with groups C and D (Table II). The resultant mortalities from mucin and saline suspension inocula are compared in Table III. Administration of *H. capsulatum* in mucin resulted in the markedly superior mortality rate of 79% as compared to that of 2.8% observed in animals receiving the organisms in saline.

Throughout the studies reported in this paper necropsies were performed on animals succumbing to the infection as well as those surviving the 30 day period. *H. capsulatum* was consistently recovered from the spleens of mice inoculated with mucin suspensions of the organism but was rarely isolated from those receiving the organisms suspended in saline.

Summary and conclusions. *H. capsulatum* in the yeast phase when suspended in 5% hog gastric mucin and administered intraperitoneally into white mice caused a high percentage of fatal infections. Equivalent challenge doses of the organism suspended in saline rarely caused death. It was observed that by varying the number of organisms administered in mucin the mortality rate as well as the time interval between injection and death could be altered to suit the experimental requirements.

Certain investigations on experimental histoplasmosis heretofore have been limited by the relative resistance of white mice to infection with *H. capsulatum*. "Virulence-enhancement" by mucin offers a means of extending the scope of such studies.

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