of inositol phosphatide, compared with a value of 72.8 dynes cm^2 for distilled water.

These observations suggest the possibility (1) that thromboplastin presents a surface which can adsorb monosodium alpha tocopherol phosphate and inositol phosphatide, (2) that the utilization of this surface by these surface-acting agents explains why their injection into mice in a solution of thromboplastin prevents the typical reaction which normally occurs in these animals following the injection of thromboplastin alone, and (3) that the addition of fibrinogen to the solution interferes with this protective action because of the surface presented by fibrinogen molecules.

Summary. Experimental studies on mice showed that the typical reaction of these animals to the injection of thromboplastin can be prevented if monosodium alpha tocopherol phosphate or inositol phosphatide is incubated with the thromboplastin before it is injected. When fibrinogen is added to the test solution, the inhibitory effect of each of these substances is lost. It is possible that alterations in surface tension may explain these phenomena.

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Use of Maxted's Method for Group Classification of Hemolytic Streptococci.*† (17715)

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Maxted(1) has recently described a method for grouping beta-hemolytic streptococci in which the streptococci are lysed by a substance, presumably an enzyme, produced by a strain of *Streptomyces albus*. Such lysates contain the streptococcal C substance and can be used as antigens in the precipitin reaction for group classification. The simplicity, reliability and usefulness of Maxted's technique have been confirmed in a study of 1,010 consecutive strains of streptococci.

Methods. The strain of *Streptomyces albus*, obtained from Maxted, was grown on the following medium which is a modification of that described previously(1).

Bacto-peptone (Difco)	0.5 g
Yeast extract, desiccated	0.6 "
Dibasic sodium phosphate, hydrous	0.2 "
Glucose	0.2 "
Fildes' extract(2)	2.5 ml
Agar	1.25 g
Distilled water	100.00 ml

These constituents, with the exception of the Fildes' extract, were mixed and dispensed into a 1000 ml Roux bottle. After sterilization at 120°C for 20 minutes, the bottle was cooled to about 40°C, the Fildes' extract added, and the agar medium allowed to gel with the bottle in the horizontal position. The surface was inoculated evenly with 1 ml of a heavy suspension of *Streptomyces albus* spores in 0.85% sodium chloride solution. After incubation at 35°C for 5 days, the culture was frozen at −10°C for 12 hours and then allowed to thaw at room temperature. This procedure separated the agar gel of the

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1. Maxted, W. R., *Lancet*, 1948, v255, 255.

2. Fildes, P., *Brit. J. Exp. Path.*, 1920, v1, 129.

medium from the fluid containing the active substance. The fluid was strained through gauze and the pH adjusted to 7.5 with 1 N hydrochloric acid. The extract was then filtered through a Seitz pad and phenol was added to a concentration of 0.5%. Preparations retained their activity for several months at 4°C. The potency of an extract was determined by its ability to lyse hemolytic streptococci. A small loopful of an 18 hour growth from a blood agar plate was inoculated into 0.25 ml of the preparation and the mixture placed in a water bath at 50°C. Lysis occurred in less than 30 minutes with very potent extracts, whereas weak preparations required as long as 24 hours.

The clear lysate, prepared with an active extract, was then used as antigen in a precipitin reaction with group specific rabbit antisera by the capillary tube technique(3). In some instances where the original suspension was very heavy and complete clearing had not occurred after long incubation (24 hours), it was possible to centrifuge the suspension and use the clear supernatant fluid as antigen. The precipitin reaction in the capillary tubes was graded according to standards previously established(3). Readings were made at the end of 1 hour of incubation at 37°C and again after overnight refrigeration at 4°C.

The 1010 consecutive strains of streptococci herein reported were isolated from the throats of normal subjects and of patients with streptococcal infections. Colonies exhibiting the *beta* type of hemolysis on medium containing 5% horse blood were transferred to medium containing 5% sheep blood. All those showing *beta* hemolysis (either on the surface or around a stab into the agar) on sheep blood medium were included in this study(4).

Results. Of the 1010 consecutive strains of hemolytic streptococci, 998 were successfully classified by Maxted's method as follows: 762 were group A, 49 group B, 94 group C, 3 group D, 4 group F, and 86 group G. There were 12 strains which could not be identified with available antisera. Nine of these 12 strains were tested with antisera for groups A, B, C, D and G. The other 3 strains were tested in addition with antisera for groups E, F, H and L. Attempts were made to confirm the results of Maxted's method by either the formamide technique(5), the hot acid method(3), or both. When the 12 strains which failed to group by Maxted's technique were examined by one or both of these methods, 11 failed to give a precipitin reaction. One strain was found to be group G by the hot acid method. This strain was not tested by the formamide method or re-examined by Maxted's technique.

Of the 998 strains which were successfully classified by Maxted's method, 791 were examined by one or both of the older methods (Table I). The first 294 strains were examined by the formamide method and 36 strains were classified by both the formamide and hot acid methods. No disagreements were obtained. An additional 461 strains were examined by the hot acid method. Of these 461 strains, 415 were successfully classified using the hot acid method and among these there were no discrepancies with the findings by Maxted's technic. Forty-six strains failed to group by the hot acid method in one attempt. These strains were lost before the tests could be repeated.

Discussion. The reliability of Maxted's method was determined by performing parallel tests on 803 strains by one of the two techniques already in general use. Whenever positive precipitin reactions were obtained by both of the methods being compared, there was complete agreement in classification. The 47 discrepancies were instances in which a positive result was obtained by one method and a negative by the other. None of these discrepancies occurred with the formamide method; all occurred when comparison was being made with the hot acid method. Experience in this laboratory with the hot acid extracts for group classification was not al-

3. Swift, H. F., Wilson, A. T., and Lancefield, R. C., *J. Exp. Med.*, 1943, v78, 127.

4. Feller, A. E., Badger, G. F., Dingle, J. H., Hodges, R. G., and Rammelkamp, C. H., *J. Clin. Invest.*, 1949, v28, 780.

5. Fuller, A. T., *Brit. J. Exp. Path.*, 1938, v19, 130.

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.