is no difference in the results obtained with media sterilized with and without the drug.

The control (+) contained approximately 20 colonies in each case. Where growth occurred in the presence

TABLE 2

Drugs added after medium was sterilized	Concentration of drug mg. per cent	Cultures 089 MA		N 360 C		71	147
		24	48	24	48	24	48
Sulfanilamide	9.4	+	+	–	–	+	+
Sodium sulfathiazole ..	9.1	–	–	–	–	–	–
Sodium sulfapyridine ..	9.1	–	–	–	–	–	–
Drugs sterilized in autoclave with medium							
Sulfanilamide	9.2	+	+	–	–	+	+
Sodium sulfathiazole ..	9.0	–	–	–	–	–	–
Sodium sulfapyridine ..	8.9	–	–	–	–	–	1 colony
Control	None	+	+	+	+	+	+

of sulfonamide (Table 2) there were fewer than 20 colonies but sufficient growth to indicate resistance to 9.2 and 9.4 mg. per cent of drug. One colony appeared in medium containing 8.9 mg. per cent of sodium sulfapyridine after 48 hours' incubation.

TABLE 3

Culture	Control		Sulfanilamide		Sodium sulfathiazole		Sodium sulfapyridine	
	24	48	24	48	24	48	24	48
1296	+	20 colonies	0	0	0	0	0	0
156	?	10 colonies	0	0	0	0	0	0
154	+	20 colonies	0	0	0	0	0	0
153	+	34 colonies	0	0	0	0	0	0
360	+	100 colonies	0	0	0	0	0	0
1172	?	2 colonies	?	6 colonies	0	0	0	6 colonies
1467	+	>100 colonies	+	>100 colonies	+	>100 colonies	+	>100 colonies
1060	+	35 colonies		30 colonies	0	0	?	20 colonies
147	+	+	0	0	0	0	0	0
089 MA	+	+	0	0	0	0	0	0

Culture 147 is, therefore, somewhat resistant to this drug.

The convenience of preparing the medium containing the drug makes routine testing of sulfonamide resistance or susceptibility a simple matter, once the medium is prepared, amounting to no more than the inoculation of one more tube and a control tube. A series of tubes containing graduated amounts of the desired sulfonamide may be prepared. For this study a concentration of approximately 10 mg. per cent in the medium has been used and has been satisfactory in most cases for determination of drug resistance or susceptibility. Other concentrations of the drug have been used to determine degree of resistance.

Table 3 shows the results of a typical day of testing with hemolytic streptococcus cultures using 10 mg. per cent concentration of the sulfonamide indicated.

Usually results can be read after 24-hour incubation. Occasionally resistance to sulfonamide activity can be seen after 48-hour incubation and would be missed if read only in 24 hours. For this reason our results are read after 24- *and* 48-hour incubation.

Inoculation of the media for these tests is done by taking a loop of an 18- to 24-hour broth culture into a tube of tryptose-phosphate broth. One loop of this dilution into the melted (and cooled) semisolid medium containing the drug will generally give 10 to 20 colonies in the control tube. The loop that we have used measures 5.0 mm. in outside diameter and is made from 28-gauge platinum wire. The loop is flamed between each inoculation to remove traces of drug and agar and to keep the size of the inoculum as constant as possible.

While our particular interest in this problem has been to devise a simple and convenient method for testing sulfonamide resistance and susceptibility of hemolytic streptococci, the method can probably be used for testing other groups of organisms.

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About the Chemical Nature of Syphilis Antigen

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In 1942 M. C. Pangborn (*3*) described a method of purifying the syphilis antigen of alcoholic beef heart extract by precipitating the phosphatides with $CdCl_2$, suspending the precipitate in petroleum ether, and eliminating the lecithin $CdCl_2$ complex by extractions with 80 per cent alcohol. The remaining solution contained cephalin $CdCl_2$ complex and antigen. After eliminating Cd by NH_3 and cephalin by precipitations with alcohol, the antigen was further purified until a substance which Pangborn claimed to be the pure syphilis antigen was obtained. According

to this author this substance, called cardiolipin, is a new, nitrogen-free, sugar-containing phospholipid, with 4.11 per cent phosphorus.

In further publications (*4, 5*) Pangborn described another method of purifying the syphilis antigen, based on the precipitation of the cephalin fraction together with the antigen by $BaCl_2$ and eliminating cephalin by alcohol. After further purification a substance was obtained with the characteristics of syphilis antigen. This second "cardiolipin" contained no sugar but approximately the same amount of phosphorus as the "cardiolipin" of the first publication.

One of the authors and his co-workers elaborated between 1931 and 1936 (cf. *1*) several methods of purifying the syphilis antigen. Having applied two of these methods successively, a purified antigen preparation was obtained recently from the phosphatid fraction of alcoholic beef heart extract. The process of purification consisted of adsorbing the antigen on specially prepared aluminum hydroxide and eluting it in benzene. The antigen was then further purified by extracting it with petroleum ether from an acidulated 80 per cent alcohol solution. From 207 grams phosphatides 1 gram of a purified antigenic preparation was obtained, with only 0.01 per cent phosphorus, 0.25 per cent reducing substances (liberated by acid hydrolysis), and 40 per cent fatty acids (liberated by alkaline hydrolysis) (*1*).

It was shown in 1936 (*2*) that by extracting an 80 per cent alcoholic solution with petroleum ether, only a part of the antigen could be obtained in the petrol ether solution, another part still remaining in the hydroalcoholic phasis. Only after acidulating this phasis with HCl did the antigen disappear completely from it, entering into the petroleum ether phasis. We may assume, therefore, that the antigen occurs in two different forms in the alcoholic organ extract, *i.e.* acid and salt.

In recent experiments we have worked on the phosphatid fraction of 10 beef hearts, obtained by extraction with alcohol and precipitation with acetone. The antigen was absorbed on aluminum hydroxide and eluted in benzene. The substance obtained in this way was dissolved in 80 per cent alcohol and extracted at first without, and then with, acidulation.

We obtained in this way two fractions, which we shall call "petroleum ether fraction before acidulation" (P.E.N.) and "petroleum ether fraction after acidulation" (P.E.A.). Both fractions reacted with strongly positive sera. P.E.N. weighed 39 mg., and no phosphorus could be detected in it. P.E.A. amounted to 22 mg. with 0.1 per cent phosphorus. Both fractions were yellow oils which hardened rapidly, forming a transparent, soft film.

We assume that the small quantities of phosphorus found in our preparations correspond to impurities, as was the case with the reducing substances in our former experiment.

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A Cheap and Speedy Method of Cleaning Old Microscope Slides

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For years our Department has been faced with the problem of cleaning off old microscope slides made with Canada balsam. None of the solvents used (xylene, toluene, turpentine, and coal oil) has proved effective in less than two weeks, and the resulting mass of dilute balsam, slides, cover slips and slide labels provided another problem in cleaning up.

Recently while collecting ectoparasites of mammals by the method recommended to me by Dr. G. H. E. Hopkins, of Uganda, which originated from Dr. F. L. Werneck, of Brazil, it occurred to me to try this upon microscope slides; it worked like a charm and is proving a boon in our laboratories.

Dr. Hopkins' method of recovering ectoparasites from fur is to place portions of hide into hot, 10-per cent caustic soda; the soda dissolves the hair and fur, and the sludge is then washed through fine, stainless steel mesh which retains the parasites. I have used this procedure extensively for parasite recovery, with considerable success, and now it is proving invaluable for cleaning slides. A number of methods can be employed, but I use three 500-cc. beakers, two of 10-per cent caustic soda on tripods over low Bunsen flames, and one spare. The caustic is kept at nearly boiling point, and a row of slides, held in a spiral of copper wire with the two ends sticking out straight, is placed across the top of the beaker so that the glass is immersed for most of its length. A large number of slides, from 1 to 13 years old have been tested and the speed of action timed; the latter varies inversely as the age and the amount of balsam present. In the case of old slides with thick balsam, the labels and cover slips slide off in from 23 to 30 seconds; in year-old slides with little balsam, it may take up to two minutes for the cover slips to fall off. All slides made with very little balsam, irrespective of age, take longer than those with much balsam. The size of cover slip does not affect the speed of action.