ferric chloride test. Therefore the dog apparently metabolizes sulfapyridine in greater part by introducing an hydroxyl group in the pyridine ring and then conjugating with a carbohydrate. This hydroxy derivative possesses some therapeutic effect in mice injected with hemolytic streptococcus and produces bacteriostasis *in vitro*, but the efficacy is less than that of sulfapyridine. As much as 80% of the diazo reacting compounds appearing in dog urine following the ingestion of sulfapyridine is in the form of this glycuronide. Only a small portion which probably is not greater than 16 percent is excreted as sulfapyridine. At least in the dog no hydroxyl group is introduced in the benzene ring and this is further verified by the fact that sulfanilamide is excreted without change.

Summary. The ingestion of sulfapyridine in the dog is followed by the appearance in the urine of a glycuronide of a hydroxy derivative of sulfapyridine. The hydroxy group is attached to the pyridine ring.

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Enhancement with Phenol of the Serological Reactivity of Lymphogranuloma Venereum Antigens.

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Three types of complement-fixing antigen prepared from yolk sac cultures of the agent of lymphogranuloma venereum have been described; *viz.*, (1) resuspended high speed sediment,[1] (2) soluble antigen in high speed supernates,[2] (3) whole suspensions inactivated with urea or ether.[3] The third type was approximately four times as active as high speed sediment.

Attempts by one of the authors (Nigg) to find a satisfactory preservative for lymphogranuloma venereum complement-fixing antigens, showed that phenol enhanced the activity of the antigen specifically since such phenolized antigens did not react with normal sera. This observation was studied further and is the subject of this note.

Table I shows the complement-fixing activity of a 10% yolk sac suspension treated with (a) 2% urea, (b) 0.25% phenol and (c) 0.5% phenol. Preparations were stored at icebox temperatures. Two separate fractions of suspension 56 when treated with 0.25% phenol failed to show increased activity when tested 8 to 30 days later. Two fractions treated with 0.5% phenol were 4 and 8 times respectively as active as the urea-treated antigen.

While 0.25% phenol had little enhancing effect at icebox temperatures, Table II shows approximately a 4-fold increase in activity when these suspensions were subjected to the following temperatures: (a) 37°C for 6 weeks (not tested earlier), (b) 56°C for 48 hours, and (c) boiling water for 10 minutes. Furthermore, the centrifuged (2,000 rpm for 10 minutes) supernates of the heated suspensions were almost as active as the whole heated suspensions. This represents a considerable purification since the slightly opalescent supernates, after removal of tissue debris and coagulated protein, were almost as active as the whole heated turbid suspensions.

That the phenol enhancement is apparently specific is shown in diagnostic tests (Table III) with positive and negative sera, comparing 2 phenolized antigens with urea-treated antigen. Antigen 57-H was treated

[1] McKee, C. M., Rake, G., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 410.

[2] Rake, G., Shaffer, M. F., Jones, H. P., and McKee, C. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 300.

[3] Nigg, C., PROC. SOC. EXP. BIOL. AND MED., **1942, 49, 132.**

TABLE I.
Effect of Phenol on the Complement-fixing Antigen of Lymphogranuloma Venereum.

Antigens: 10% suspensions treated with:		L.V. serum pool 12 (1-60) Antigen dilutions: 1-200	1-400	1-800	1-1600	1-3200	1-6400	Interval between preparation and testing, days
2% urea:	56-1	4+	4+w	1+	0			
0.25% phenol:	56-2	4+	4+	1-2+	0			30
	56-5	4+	4+	3-4+	tr			8
0.5% phenol	56-7B	4+	4+	4+	4+			8
	"	4+	4+	4+	4+	2+	0	37
	56-8	4+	4+	4+	4+	2+		7
	"	4+	4+	4+	4+	4+	2+	28

Results are stated in terms of the degree of fixation.

TABLE II.
Effect of Temperature on Phenolized Complement-fixing Antigens of Lymphogranuloma Venereum.

Antigens: 10% suspensions treated with:	Temperature exposure		L.V. serum pool 12 (1-60) Antigen dilutions: 1-200	1-400	1-800	1-1600	1-3200	1-6400
2% urea:								
56-1	Icebox 8 wk		4+	4+	1+	0		
0.25% phenol:								
56-2	Icebox 6 wk		4+	4+	3+w	0		
"	Room temperature 6 wk		4+	4+	3+w	0		
"	37°C 6 wk		4+	4+	4+	4+	1-2+	
2% urea:								
57-1	Icebox 5 wk		4+	4+w	0			
0.25% phenol:								
57-F-5a	56°C 48 hr	Whole susp	4+	4+	4+	3-4+	1-2+	0
		Supernate	4+	4+	4+w	1+	0	0
57-F-5b	Boiled 10 min.	Whole susp.	4+	4+	4+	4+	1-2+	0
		Supernate	4+	4+	4+	4+w	1+	0

with 0.25% phenol at 56°C for 18 hours. Antigen 57-F-6a is the slightly opalescent supernate from a boiled suspension likewise containing 0.25% phenol. These 3 antigens were prepared from the same initial 10% yolk sac suspension. One unit of each antigen, determined by preliminary titration, was used. The enhancement is apparently specific since suspensions of normal yolk sac treated in identical manner do not give fixation even in a 1-250 dilution. The anticomplementary activity of all antigens and sera was ruled out by appropriate controls.

Discussion. The enhanced complement-fixing activity of phenolized suspensions is of considerable practical significance; *viz.*, (1) the activity is increased from 4 to 8 times, (2) objectionable turbidity is removed, and (3) phenol acts as an effective preservative without the deleterious effect produced by formalin, *e.g.*,[3] and (4) thermostability facilitates shipping these antigens without refrigeration and their use in warm climates.

Information has also been obtained regarding the nature of the complement-fixing substance, *viz.*, (1) its stability at boiling temperature, (2) its activation by phenol and (3) its properties which are not characteristic of native proteins. Studies on the chemical nature of the active substance are in progress.

In a limited number of tests with sera from early syphilis non-specific fixation was never encountered with the phenolized antigens, whereas it was frequently observed with both the resuspended high speed sediment[4] and urea-inactivated antigens.[5]

[4] Shaffer, M. F., Rake, G., Grace, A. W., McKee, C. M., and Jones, H. P., *Am. J. Syph., Gon., and Ven. Dis.*, 1941, **25**, 699.

[5] Nigg, C., unpublished data.

TABLE III.
Comparison of Phenolized Antigens with Urea Antigen in Diagnostic Tests for Lymphogranuloma venereum.

Sera	Antigen 57-H treated with 0.25% phenol at 56°C 18 hr (1-1000) Serum dilutions: 1-5	1-10	1-20	1-40	1-80	1-160	Control antigen 11-C treated with 0.25% phenol at 56°C 18 hr (1-1000) *
13			4+	4+	4+	2+	0
Re			4+	4+	4+	2-3+	0
Ga	4+w	2-3+	0				0
BB	0						0
	Supernate of antigen 57-F-6a treated with 0.25% phenol, boiled 10 min (1-1000)						Supernate of control antigen 11-B treated with 0.25% phenol, boiled 10 min (1-1000)
13			4+	4+	4+	3-4+	0
Re			4+	4+	4+	3-4+	0
Ga	3-4+	2+	0				0
BB	0						0
	Antigen 57-1 treated with 2% urea (1-200)						Control antigen 9-10 treated with 2% urea (1-200)
13			4+	4+	4+w	3-4+	0
Re			4+	4+	4+	3-4+	0
Ga	3-4+	2+	0				0
BB	0						0

* The lowest dilution of serum which was used with the specific antigen was used in testing the control antigens which were prepared from normal yolk sacs.

The purified phenolized antigens may possibly eliminate, to a certain extent at least, the cross reactions which preclude differential serological diagnosis in the lymphogranuloma-psittacosis group.[6-10] Similar enhancement with phenol might obtain with antigens of other members of this group.

The identity of the skin-reactive and complement-fixing antigens is being investigated in coöperation with Dr. A. W. Grace, by comparing in Frei tests the purified phenolized antigens with other yolk sac antigens.

The exact conditions which produce phenol enhancement are not yet thoroughly understood. Occasional preparations have been encountered in which enhancement was comparatively slight with the same concentration of phenol which regularly gave marked enhancement of other preparations. That the pH influences the degree of enhancement is indicated by preliminary experiments.

6 Rake, G., Eaton, M. D., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 528.

7 Eaton, M. D., Martin, W. P., and Beck, M. D., *J. Exp. Med.*, 1942, **75**, 21.

8 Eddie, B., and Francis, T., Jr., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 291.

9 Rake, G., Jones, H. P., and Nigg, C., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 449.

10 Levine, S., Holder, E. C., and Bullowa, J. G. M., *J. Immunol.*, 1943, **46**, 183.