TABLE II.
Local Treatment of Mice Injected Intramuscularly with *Cl. perfringens.*

Drug	Mg inj.	No. of tests	No. of mice	No. lived	No. died	% lived
NaSD*	1.0	5	40	37	3	92
"	0.1	7	60	52	8	87
132	1.0	3	30	23	7	77
"	0.5	6	60	35	25	58
617	1.0	3	25	10	15	40
627	1.0	3	25	9	16	36
654†	1.0	3	30	12	18	40
684	1.0	4	40	28	12	70
"	0.5	5	50	32	18	64
690	1.0	3	30	10	20	33
713†	1.0	4	40	2	38	5
" †	0.5	3	30	15	15	50
Controls	—	17	150	6	144	4

* Sodium sulfadiazine.
† Suspended in acacia.

1% in the diet, a slight degree of protection against *Strep. hemolyticus,* but not against *Staph. aureus,* was obtained. 132 at 1% in the diet did not protect mice infected with *Cl. perfringens,* although 1% sulfathiazole protected 50%. That these results were not due to non-absorption of 132 was shown by detection of this drug in the urine by means of *in vitro* tests with *Cl. perfringens.*

Three of the compounds were tested for bacteriostatic action on 15 strains of staphylococci by a serial dilution test in charcoal-adsorbed beef heart infusion broth, containing very little *p*-amino benzoic acid.[12] At 6.2 mg % all the strains were inhibited by 132 and 684 for 10 hours, although some grew in 17 hours. 132 and 684 also prevented growth of all strains for 17 hours when present in a concentration of 25 mg %, although by 57 hours all strains had grown. 690 had no demonstrable action.

[12] MacLeod, C. M., and Mirick, G. S., *J. Bact.,* 1942, **44**, 277.

Summary. Except for sulfadiazine, homosulfanilamide and its N^1-methyl derivative were the most effective drugs in local treatment of experimental gas gangrene of the compounds tested. The acute toxicity of homosulfanilamide was less than that of its derivative. The chronic toxicity of homosulfanilamide in mice appeared to be no greater than that of sulfanilamide. These two compounds were not effective in the treatment of infections in mice with *Strep. hemolyticus* C203, *H. influenzæ* 62/B, or *Staph. aureus* Smith, but they were bacteriostatic *in vitro* for staphylococci. Although infection with *Cl. perfringens* in mice is not strictly comparable with infection in man, these results indicate that homosulfanilamide may be worthy of a clinical trial in the local treatment of gas gangrene.

14506

Differentiation Between Fievre Boutonneuse and Rocky Mountain Spotted Fever by Means of Complement Fixation.

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It has been shown by Badger[1] that Fievre Boutonneuse, a disease of the Mediterranean basin transmitted by *Rhipicephalus sanguineus* ticks, and Rocky Mountain spotted fever are related, for one disease protects

[1] Badger, L. F., *Public Health Rep.,* 1933, **48**, 507.

against the other in cross immunity tests in guinea pigs. On the other hand, Davis and Parker[2] have shown that a vaccine prepared from inactivated Rocky Mountain spotted fever rickettsiæ (tick vaccine) which protects against Rocky Mountain spotted fever affords no protection against Fievre Boutonneuse. While the reverse has not been demonstrated, namely, whether a vaccine prepared from inactivated Fievre Boutonneuse rickettsiæ confers an immunity to Rocky Mountain spotted fever, the work referred to indicates that while there is a group relationship between these diseases, there are some antigenic differences.

Because of the cross immunity conferred in guinea pigs by these two diseases laboratory differentiation may be difficult. The following communication presents data to indicate that these two diseases can be differentiated by means of specific complement fixation.

The antigen for the complement fixation test in Rocky Mountain spotted fever is best prepared from tissue cultures on agar.[3] This antigen is specific and with it Rocky Mountain spotted fever can be differentiated from either epidemic or murine typhus fever. This method is used routinely in the Division of Virus and Rickettsial Diseases at the Army Medical School for the diagnosis of Rocky Mountain spotted fever.

The antigen for Fievre Boutonneuse is prepared in the identical manner as those described for epidemic and murine typhus.[4] The antigen is prepared from yolk sac cultures from fertile hens' eggs. The infected yolk sacs are harvested and inactivated with 0.5% formalin. The rickettsiæ are extracted and further purified by centrifugation in the cold in an angle centrifuge at 4,000 r.p.m. for one hour and washed 4 times in buffered saline, pH 7.0. In this instance, as with the typhus antigens, it was found that a soluble antigen is liberated in the supernatant fluid. The soluble antigen obtained from typhus rickettsiæ gives cross fixation with epidemic and murine convalescent serum while the soluble antigen obtained from Fievre Boutonneuse cultures gives cross fixation with Rocky Mountain spotted fever convalescent serum. (Protocol III). A soluble antigen is also found in agar tissue cultures of Rocky Mountain spotted fever.[5] In both instances however the washed rickettsial bodies provide a specific antigen.

The method of performing the tests has been previously described.[3,4]

Protocol I presents the results obtained with convalescent guinea pig serum from animals recovered from Fievre Boutonneuse, Rocky Mountain spotted fever, epidemic and murine typhus using purified Fievre Boutonneuse and Rocky Mountain spotted fever rickettsial antigens.

This experiment demonstrates that specific complement fixation can be obtained with guinea pig convalescent Fievre Boutonneuse or Rocky Mountain spotted fever serum when the homologous antigen is used but no fixation is obtained with the heterologous antigens. Convalescent epidemic typhus, murine typhus, and normal guinea pig serum gives no fixation with either of these antigens.

Protocol II shows the results obtained with human Fievre Boutonneuse and Rocky Mountain spotted fever convalescent serum using purified Fievre Boutonneuse and Rocky Mountain spotted fever rickettsial antigens.

All specimens were negative using an epidemic or murine typhus antigen. Likewise, 5 human epidemic typhus and 5 murine typhus convalescent specimens were negative using a Fievre Boutonneuse or Rocky Mountain spotted fever antigen.

The results indicate that with Fievre Boutonneuse convalescent serum there is some cross fixation in low titer, but a higher titer is obtained with the homologous antigen. It is of interest to note that the highest titer occurred in a specimen of serum obtained from the most recent case (1 year) but a significant titer persisted 10 years after an attack of Fievre Boutonneuse.

With Rocky Mountain spotted fever convalescent serum some cross fixation is obtained

[2] Davis, G., and Parker, R. R., *Pub. Health Rep.*, **1934, 49,** 423.

[3] Plotz, H., and Wertman, K., *Science,* 1942, **95, 441.**

[4] Plotz, H., *Science,* 1943, **97,** 20.

[5] Unpublished data.

PROTOCOL I.
Reactions with Convalescent Guinea Pig Serum (Bled 2 weeks after infection).

Disease	Guinea pig No.	Serum titer with F.B. antigen*	Serum titer with R.M.S.F. antigen*
Fievre Boutonneuse	1889	1/20	0
	1890	1/20	0
	1891	1/80	0
	1895	1/160	0
	1897	1/80	0
	1898	1/80	0
Rocky Mountain spotted fever	Pool A	0	1/320
	" B	0	1/80
	" C	0	1/80
Epidemic typhus	4 pools; C.F. positive for epidemic typhus	0,0,0,0	0,0,0,0
Murine typhus	4 pools; C.F. positive for murine typhus	0,0,0,0	0,0,0,0

* Only 4 and 3 plus reactions read as end-point titers.

PROTOCOL II.
Reactions with Human Fievre Boutonneuse and Rocky Mountain Spotted Fever Convalescent Serum.

Disease	Case No.	Time after onset	Serum titer with F.B. antigen*	Serum titer with R.M.S.F. antigen*
F.B.	1	1 yr	1/320	1/20
	2	2 "	1/40	0
	3	5 "	1/40	0
	4	6 "	1/80	1/10
	5	10 "	1/20	0
R.M.S.F.	1	140 days	1/40	1/640
	2	31 "	1/160	1/640
	3	217 "	0	1/80
	4	14 "	0	1/160
	5	29 "	0	1/80
	6	146 "	0	1/80
	7	30 "	1/10	1/1280
	8	57 " (1st spec.)	1/10	1/320
		120 " (2nd ")	1/20	1/640
	9	5 " (1st ")	0	0
		64 " (2nd ")	1/20	1/320
	10	75 " (1st ")	1/10	1/320
		155 " (2nd ")	1/20	1/640

* Initial dilution of serum.

with a Fievre Boutonneuse antigen thus demonstrating antigenic relationship. In all cases, however, when cross fixation does occur in Rocky Mountain spotted fever the titer obtained with a Rocky Mountain spotted fever antigen was higher than was obtained with the Fievre Boutonneuse antigen. In those cases where specimens were obtained at two periods during the course of the disease a rise in antibody titer was observed with the homologous antigen, but no significant rise occurred with the Fievre Boutonneuse antigen.

As was previously pointed out the soluble antigen obtained from washings of Fievre Boutonneuse yolk sac cultures gives cross fixation with Rocky Mountain spotted fever convalescent guinea pig serum. Protocol III demonstrates this point. The same Rocky Mountain spotted fever pooled serum which gave fixation with the washings obtained from the Fievre Boutonneuse cultures gave negative results when subsequently tested with the purified Fievre Boutonneuse rickettsial antigen.

Summary. Data are presented to indicate that Fievre Boutonneuse can be differentiated from Rocky Mountain spotted fever by means of complement fixation provided purified

PROTOCOL III.
Fievre Boutonneuse Supernatant (4,000 R.P.M.)

Antigen dil.*	1/10	1/20	1/40	1/80	1/160	1/320	1/640	Serum control 1/10
Serum Dilutions—Fievre Boutonneuse Convalescent G.P. Pool.								
1/3	4	4	4	4	3	2	1	0
1/4	4	3	2	1	0	0	0	
1/6	1	0	0	0	0	0	0	
1/8	0	0	0	0	0	0	0	
Serum Dilutions—Rocky Mountain Spotted Fever G.P. Pool.								
1/3	4	4	4	4	4	4	4	0
1/4	4	4	4	4	3	3–	2	
1/6	4	3	1	0	0	0	0	
1/8	2	1	0	0	0	0	0	
Serum Dilutions—Normal G.P. Pool.								
1/3	2	1	0	0	0	0	0	0
1/4	0	0	0	0	0	0	0	
1/6	0	0	0	0	0	0	0	
1/8	0	0	0	0	0	0	0	

* Antigen anticomplementary at 1/2 dilution.

rickettsial antigens are used. This test may be employed with convalescent guinea pig or human serum thus providing a convenient laboratory test for diagnosing Fievre Boutonneuse. Since there is some cross fixation between Fievre Boutonneuse and Rocky Mountain spotted fever further evidence is presented to indicate that these diseases are antigenically related. Other members of the Spotted Fever Group are now being similarly studied.

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14507 P

Development of Tumors in the Rat Ovary After Transplantation into the Spleen.

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It has been shown by Golden and Sevringhaus[1] that rats in which the ovaries have been transplanted to the mesentery remain anestrous, owing to inactivation in the liver (previously reported by Zondek[2]) of the estrogen thus secreted into the portal circulation.

This phenomenon was more extensively studied by G. R. Biskind[3-6] using pellets of crystalline steroids implanted in another organ in the portal circulation, the spleen. Castrate

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1 Golden, June B., and Sevringhaus, E. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 361.

2 Zondek, B., *Skandinav. Arch. f. Physiol.*, 1934, **70**, 133.

3 Biskind, G. R., and Mark, J., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 212.

4 Biskind, G. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 259.

5 Biskind, G. R. *Endocrinology*, 1941, **28**, 218.

6 Biskind, G. R., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 452.