TABLE II.
mg %.

Days after implantation of tablets	Chloride				Sodium			
	I Contr.	II Estrog.	III Estr.-Deso.	IV Deso.	I Contr.	II Estrog.	III Estr.-Deso.	IV Deso.
45	360	329	346	318	277	278	263	274
73	352	351	376	380	277	279	287	290
101	381	374	373	368	278	278	314	295

Days after implantation of tablets	Potassium			
	I Contr.	II Estrog.	III Estr.-Deso.	IV Deso.
45	31.4	25.5	22.7	31.7
73	34.0	27.3	28.0	29.9
101	19.7	21.8	19.8	26.9

was rather high in Groups III and IV, but the average was not higher than the maximal values in Groups I and II without desoxycorticosterone. There were important variations of potassium, but they were not related to the administration of desoxycorticosterone.

Summary. A group of castrated female guinea pigs was treated with fibromatogenic quantities of diethylstilbestrol; another group was treated simultaneously with diethylstilbestrol and quantities of desoxycorticosterone acetate sufficient to prevent estrogen-induced abdominal fibroids. The average fibrous tumoral effect diminished by the action of desoxycorticosterone from 5.5 to 1.7 units. Concentration of chloride, sodium and potassium in the blood of the groups treated with diethylstilbestrol, desoxycorticosterone or both was within the same range as in animals not treated. These results show that the antifibromatogenic quantity of desoxycorticosterone acetate is smaller than those doses which might interfere with the concentration of chloride, sodium or potassium in the blood.

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Antigenic Differences Between Strains of Scrub Typhus as Demonstrated by Cross-Neutralization Tests.

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The question of whether antigenic differences occur among strains of scrub typhus is important not only from an epidemiological point of view but also with regard to the development of a vaccine for its control. Epidemiologic studies have indicated a marked variation in the severity of outbreaks of this disease in different geographical areas.[1,2] Furthermore, there have been undocumented rumors from medical officers returning from the southwest Pacific area that individuals recently recovered from the disease in one locality have been reinfected in another. This

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[1] Blake, F. G., Maxey, K. F., Sadusk, J. F., Jr., Kohls, G. M., and Bell, E. J., *Am. J. Hyg.*, 1945, **41**, 243.

[2] Kohls, G. M., Armbrust, C. A., Irons, E. N., and Philip, C. B., *Am. J. Hyg.*, 1945, **41**, 374.

would imply that the immunity conferred by the first infection was inadequate to prevent the second. However, such an inadequacy might be attributed as easily to relative pathogenicity of strains as to antigenic differences.

Although experimental evidence indicates that laboratory animals which survive infection with one strain are completely immune to infection with other strains,[1,3,4] this by itself is not conclusive proof of the complete antigenic identity of such strains. For example, guinea pigs recovered from an infection with either epidemic or murine typhus are resistant to infection not only with the homologous agent but also with the heterologous agent. Nevertheless, antigenic differences between the agents are readily demonstrable by serological means[5] and the vaccination of animals with one of them gives little protection against infection with the other.[6] A similar relationship probably exists between strains of scrub typhus. Thus, Topping[3] showed that infection with the Karp or Gilliam strain conferred immunity to the heterologous as well as homologous strains while Bengtson[7] presented evidence of their antigenic differentiation. She found[7] that sera from patients and animals infected with the Karp or Gilliam strain fixed complement strongly with the homologous antigen but only weakly with the heterologous. Not all strains are thus separable, for Smadel, Rights and Jackson[4] found the Imphal and Calcutta strains to be indistinguishable in cross-immunity and complement fixation tests.

The data to be presented indicate that differentiation between certain strains of *R. orientalis* can be demonstrated by means of cross-neutralization tests.

Strains of Rickettsia orientalis. Three strains of *R. orientalis* were used in the present experiments. The Kostival strain[1] was isolated in the Dobadura area, New Guinea, from a case of naturally acquired infection. The Host 21 strain,[1] recovered from mites *(T. jletcheri)* taken from a wild caught bandicoot *(Echymipera cockerelli)*, was also isolated in the Dobadura area, New Guinea. Both strains have been maintained by passage in mice and in yolk sacs of embryonated eggs. The Seerangayee strain[8] was isolated by Lewthwaite and Savoor from a case occurring in southern Malaya and has since been maintained in mice and guinea pigs.

Preparation of Sera. Kostival and Seerangayee antisera were prepared by inoculating rabbits with a 10 or 20% suspension of liver and spleen from infected hamsters. 5.0 cc were injected intraperitoneally and 2.0 cc subcutaneously on 2 occasions a week apart. Two weeks after the second injection the rabbits were bled and their sera pooled. The Host 21 antiserum consisted of pooled sera from guinea pigs which had received a single intraperitoneal injection of 2.0 cc of a 10% suspension of infected hamster liver and spleen. The guinea pigs were exsanguinated one month after the inoculation.

Neutralization Tests. The technic used for the performance of neutralization tests consisted of adding undiluted experimental and control sera to equal volumes of 10-fold dilutions of previously standardized stock rickettsial suspensions. These suspensions were prepared from infected yolk sacs or mouse tissues and stored at —70°C. The dilutions of infectious material were $10^{-1.7}$, $10^{-2.7}$, etc., so that after the addition of the serum the final dilutions would be 10^{-2} (1:100), 10^{-3} (1:1000), etc. After mixing, the tubes were placed in ice water and 0.2 cc amounts of each of the serum-virus mixtures were injected intraperitoneally into 5 to 10 mice. Mice were observed daily for 21 days at which time the survivors were discarded. The LD_{50} titers of the virus in the experimental and control sera were determined by the method of Reed and Muench, the values expressed as the logarithm of the reciprocal

[3] Topping, N. H., *Pub. Health Rep.*, 1945, **60**, 945.

[4] Smadel, J. E., Rights, F. L., and Jackson, E. B., *J. Exp. Med.*, 1946, **83**, 133.

[5] Plotz, H., Wertman, K., and Bennett, B. L., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 76.

[6] (a) Ventemillas, F., *J. Immunol.*, 1939, **36**, 339; (b) Wertman, K., unpublished data.

[7] Bengtson, I. A., *Pub. Health Rep.*, 1945, **60**, 1483.

[8] Lewthwaite, R., and Savoor, S. R., *Brit. J. Exp. Path.*, 1936, **17**, 1.

TABLE I.
Titration of Kostival Strain in the Presence of Homologous and Heterologous Rabbit Immune Serum.

Immune serum	Dilution of rickettsiae 10-2	10-3	10-4	10-5	10-6	10-7	10-8	Infectious titer† (log)	Neutralizing index† (log)
Kostival rabbit No. 2	0/6*	0/6	2/6	1/6	0/6	—	—	2.0	4.2
Seerangayee rabbit No. 1	6/6	6/6	3/6	4/6	2/6	—	—	5.1	1.1
Normal rabbit	6/6	5/6	6/6	5/6	3/6	3/6	0/6	6.2	—

* Numerator = number of mice dying.
Denominator = number of mice inoculated.
† Titers are recorded as the logarithm of the reciprocal of the dilution of virus producing 50% mortality. The index is recorded as the difference between the logarithmic exponents of the titers of the virus in the experimental and control sera.

TABLE II.
Preliminary Cross-Neutralization Tests Between Kostival, Seerangayee and Host 21 Antisera and Their Respective Agents.

Exp. No.	Infectious agent	Immune serum	Infectious titer in Test group (log)	Infectious titer in Control group (log)	Neutralizing index (log)
1	Kostival	Kostival rabbit No. 1	2.0	5.8	3.8
2	Host 21	" "	4.0	3.4	—0.6
3	Kostival	" "	2.0	6.5	4.5
	Host 21	" "	5.4	6.2	0.8
4	Kostival	Kostival rabbit No. 2	2.0	6.2	4.2
	"	Seerangayee rabbit No. 1	5.1	6.2	1.1
5	Seerangayee	" "	2.0	6.3	4.3
6	Host 21	Host 21 GP No. 1	2.2	6.5	4.3
	Kostival	" "	3.3	6.5	3.2

See explanatory notes of Table I.

of the calculated dilution producing mortality in 50% of the animals inoculated. The neutralizing index was taken as the difference between the logarithmic exponents of the experimental and control titers. An example of such a titration is given in Table I. The detailed data are from Experiment 4, which is summarized in Table II.

Results. The results of a series of exploratory experiments are summarized in Table II. In Experiment 1 the Kostival antiserum was shown to neutralize the homologous agent. In Experiment 2, however, this serum failed to neutralize the heterologous strain (Host 21). A fresh Host 21 suspension was prepared and used in Experiment 3. Here the Kostival antiserum again neutralized the homologous strain but failed to neutralize the heterologous strain. In Experiment 4 a new Kostival antiserum was utilized and compared with a Seerangayee antiserum with results similar to those obtained above, namely, the homologous serum neutralized the agent while the heterologous serum was relatively inert. That this was not due to lack of antibodies in the Seerangayee antiserum is indicated by Experiment 5 where excellent homologous neutralization was demonstrated. In Experiment 6, however, a somewhat different situation was encountered in that the Host 21 guinea pig antiserum, which neutralized its own virus to high titer, also neutralized the heterologous Kostival strain.

Subsequently, a series of experiments were performed in which 3 of the antisera used in the earlier studies were tested against the 3 agents. The results summarized in Table III indicate that, as before, the Kostival antiserum was effective against the Kostival strain only. Similarly, the Seerangayee antiserum was relatively ineffective against both the heterologous strains. In contrast, the Host 21 antiserum was capable of neutralizing all 3 strains, in fact, giving a higher index against

TABLE III.
Cross-Neutralization Tests Between Kostival, Seerangayee and Host 21 Antisera and Their Respective Agents.

Exp. No.	Infectious agent	Immune serum	Infectious titer in Test group (log)	Infectious titer in Control group (log)	Neutralizing index (log)
7	Kostival	Kostival rabbit No. 2	3.3	6.0	2.7
	"	Seerangayee rabbit No. 1	5.1	6.0	0.9
	"	Host 21 GP No. 1	3.7	6.0	2.3
8	Seerangayee	Kostival rabbit No. 2	6.2	7.2	1.0
	"	Seerangayee rabbit No. 1	3.6	7.2	3.6
	"	Host 21 GP No. 1	3.5	7.2	3.7
9	Host 21	Kostival rabbit No. 2	5.4	5.7	0.3
	"	Seerangayee rabbit No. 1	4.2	5.7	1.5
	"	Host 21 GP No. 1	3.4	5.7	2.3

See explanatory notes of Table I.

the Seerangayee virus than against its own Host 21 strain. The significance of this fact remains to be determined. It is to be noted that all homologous serum titers had dropped considerably since the experiments recorded in Table II. It is possible that this was due to technical details in performing the test which have escaped our notice, or that it was related to the fact that the sera were stored at 4°C. Workers in the virus field recognize that the antibody titers of certain sera diminish when stored at 4°C. Attempts to restore the activity of scrub typhus sera by the addition of freshly drawn rabbit or guinea pig serum were unsuccessful; this was in contrast to the results of Morgan[9] and Whitman[10] in reactivation experiments with Western equine encephalitis antisera.

Discussion. The experiments reported above indicate that distinct differences can be demonstrated between certain strains of *R. orientalis* by means of cross-neutralization tests. It is of interest that while the Kostival strain from the Dobadura area of New Guinea produced a serum which had little capacity to neutralize the Host 21 strain obtained from the same area, yet the serum from guinea pigs inoculated with Host 21 had considerable capacity to neutralize the Kostival agent. A similar situation existed between the Seerangayee strain from Malaya and the Host 21 strain. Several possibilities arise to explain such a phenomenon. In the first place, the origin of the strains may be responsible for these differences. Host 21 was recovered from mites taken from a wild caught bandicoot *(Echymipera cockerelli)* whereas Kostival and Seerangayee were isolated from human cases. Perhaps the parent mite strains are antigenically more complete than those isolated from humans. Secondly, both the Kostival strain and the Seerangayee strain had been passaged either through chick embryo (Kostival) or guinea pigs (Seerangayee) with much greater frequency than the Host 21 strain. This may have resulted in a partial loss of antigenic structure. Thirdly, the Host 21 antiserum was prepared in guinea pigs, whereas the other 2 antisera were obtained from rabbits. Although experiments have indicated no difference between normal guinea pig and normal rabbit serum in virucidal properties, it is possible that this difference in animal species may play an important part in the specificity of the sera produced.

Summary and Conclusions. The antigenic relationship of 3 strains of *R. orientalis* was studied by means of cross-neutralization tests. Antiserum produced in rabbits to the Kostival or Seerangayee strains neutralized the homologous strain only. However, antiserum produced in guinea pigs to the Host 21 strain showed a broader coverage.

[9] Morgan, I. M., *J. Immunol.*, 1945, **50**, 359.

[10] Whitman, L., to be published.