

Likewise, antiserum 2-193 agglutinated strain 205 to titer, and when adsorbed with 205 had a residual homologous titer of only 40.

It thus appears that antiserum P143 may

be rendered more nearly monovalent by adsorption with *Sh. dispar*, type I and IIa or IIc. This procedure would prevent confusion in classification of cultures such as 2-193.

15287

Immunity Following Para-Aminobenzoic Acid Therapy in Experimental Tsutsugamushi Disease (Scrub Typhus).

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(Introduced by S. Bayne-Jones.)

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Two recent communications^{1,2} from this laboratory have dealt with the therapeutic effectiveness of para-aminobenzoic acid (PABA) in the treatment of experimental tsutsugamushi disease. The beneficial effect of PABA and especially that of the sodium salt of the acid (NaPAB) was demonstrated against the Ceylon, Calcutta, and Karp strains of *R. orientalis*^{1,2} and later against an Imphal strain.³ The animals used in these experiments were 2 species of desert rodents, *Gerbillus pyramidum* and *Gerbillus gerbillus*, which are abundant in Egypt.⁴ The susceptibility of gerbilles to these strains of *R. orientalis* and the manifestations of the disease in them have been detailed elsewhere.⁵

Para-aminobenzoic acid has also been shown experimentally to have a beneficial therapeutic effect on murine⁶⁻⁹ and epidemic typhus,⁷

and on Rocky Mountain spotted fever.^{10,11} Clinical trial of PABA has demonstrated it to be of value in the treatment of louse-borne typhus fever.¹² There has also been recently reported a case of Rocky Mountain spotted fever which received PABA with apparent benefit.¹³

Thus, it has been shown by several investigators that PABA has an effect on several rickettsial agents. The mode of action of PABA on these organisms is of great interest since they are obligate intracellular parasites. Various studies carried out in developing chick embryos indicate that PABA is rickettsiostatic rather than rickettsiocidal.⁷⁻¹⁰ Furthermore, in PABA treated human cases of epidemic typhus fever and the case of Rocky Mountain spotted fever there was no apparent disturbance of the antibody dynamics as judged from findings in Weil-Felix and complement-fixation

¹ Snyder, J. C., and Zarafonetis, C. J. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1945, **60**, 115.

² Murray, E. S., Zarafonetis, C. J. D., and Snyder, J. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1945, **60**, 80.

³ Unpublished observations of the authors.

⁴ Snyder, J. C., Zarafonetis, C. J. D., and Lui, W. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1945, **59**, 110.

⁵ Zarafonetis, C. J. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1945, **59**, 113.

⁶ Snyder, J. C., Maier, J., and Anderson, C. R., Report to the Division of Medical Sciences, National Research Council, Washington, D. C., December 26, 1942.

⁷ Hamilton, H. L., Plotz, H., and Smadel, J. E.,

PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 255.

⁸ Greiff, D., Pinkerton, H., and Moragues, V., *J. Exp. Med.*, 1944, **80**, 561.

⁹ Greiff, D., and Pinkerton, H., *J. Exp. Med.*, 1945, **82**, 193.

¹⁰ Hamilton, H. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1945, **59**, 220.

¹¹ Anigstein, L., and Bader, M. N., *Science*, 1945, **101**, 591.

¹² Yeomans, A., Snyder, J. C., Murray, E. S., Zarafonetis, C. J. D., Ecke, R. S., *J. A. M. A.*, 1944, **126**, 349.

¹³ Rose, H. M., Duane, R. B., and Fischel, E. E., *J. A. M. A.*, 1945, **129**, 1160.

tests.^{12,13} These observations support the previously advanced hypothesis that PABA inhibits the multiplication of rickettsiae inside the cells, thereby permitting the immunity mechanisms of the body to dispose of them.¹² The treatment of these rickettsial infections with PABA, therefore, should not interfere with the development of immunity to them. This has been shown to be true in guinea pig experiments with Rocky Mountain spotted fever.¹³ In order to obtain additional data, however, immunity tests were carried out in gerbilles which had survived inoculation with scrub typhus with the aid of PABA therapy. The purpose of this report is to give the results of these tests.

Immunity Tests. Six to 9 months after the PABA therapy experiments were concluded, there were 69 treated gerbilles available for immunity tests. Of these, 24 were from the Ceylon strain experiments, 4 from the Calcutta, 32 from the Karp, and 9 from the Imphal strain experiment. The gerbilles had gained weight and appeared healthy at the time of testing for immunity to their respective homologous strains.

The test inocula consisted of peritoneal washings from one or more gerbilles which had been infected with the strain desired. Three cubic centimeters of sterile physiological saline solution were used to make the peritoneal washings, and, from this, serial ten-fold dilutions were made. Each gerbille was inoculated intraperitoneally with 1 cc of the 10^{-2} dilution of homologous strain peritoneal washings. Simultaneously, each strain inoculum was titrated in normal gerbilles in dilutions from 10^{-1} through 10^{-6} . The animals were then observed for 21 days, at which time the experiment was arbitrarily closed.

There was but one death in the 69 gerbilles tested, though the animals had received from 2,140 to over 3,600 lethal doses of infectious material. The single death occurred in a gerbille of the Karp strain group. It died on the 11th post-inoculation day and autopsy was not at all suggestive of scrub typhus. The liver and spleen appeared normal and there was no pleural effusion. Large numbers of bacteria were seen in smears of

the peritoneal exudate.

It appears, then, that these gerbilles which had been infected with scrub typhus and survived through treatment with PABA developed a strong immunity to the homologous strain of *R. orientalis*. It should be noted, however, that Fox and Peterson have found that *R. orientalis* persist up to a year or more in mice which survived either as a result of methylene blue treatment or as a result of infection by subcutaneous inoculation.¹⁴ The presence of active organisms in the cells of an infected animal might well give rise to "interference phenomenon" so that subsequent survival to re-inoculation might not represent immunity in the usual sense of the term. The presence of surviving rickettsiae from primary inoculation was not investigated in the gerbilles used in these tests. However, the fact that the gerbilles resisted challenge indicates that infection was established on primary inoculation in spite of PABA treatment. If immunity developed in these gerbilles and is the reason for their survival, then, the effect of PABA was to inhibit the rickettsiae permitting the mechanisms of immunity to develop. Should living *R. orientalis* be demonstrated in animals surviving infection with the aid of PABA treatment as it has been in the case of methylene blue treated mice, the conclusion would again be that the effect of PABA was rickettsiostatic rather than rickettsiocidal.

Cross-immunity Tests. Cross-immunity between various strains of *R. orientalis* has been demonstrated on several occasions. The literature on this subject has been recently reviewed by Blake, *et al.*¹⁵ who also investigated cross-immunity with other strains. Additional strains have since been tested by other workers.¹⁶ However, cross-immunity has not been described for the 4 strains involved in these PABA studies. Therefore, upon the completion of the immunity tests

¹⁴ Fox, J., and Peterson, O., personal communication.

¹⁵ Blake, F. G., Maxey, K. F., Sadusk, J. F., Jr., Kohls, G. M., and Bell, E. J., *Am. J. Hyg.*, 1945, **41**, 243.

¹⁶ Kohls, G. M., Armbrust, C. A., Irons, E. N., and Philip, C. B., *Am. J. Hyg.*, 1945, **41**, 374.

of the PABA treated gerbilles with their respective homologous strains of *R. orientalis*, cross-immunity tests were performed with heterologous strains. Inocula were prepared and administered in the same manner as described for the immunity tests. Simultaneous titrations of the infectious material were made in normal gerbilles with dilutions of 10^{-1} through 10^{-8} . The test period of 21 days remained the same.

Of the 24 gerbilles immune to the Ceylon strain, 9 were tested with the Karp strain, 8 with the Calcutta, and 7 with the Imphal strain. One death occurred in the group challenged with the Calcutta strain, 15 days after the inoculation. This gerbille showed evidence of marked weight loss, but the autopsy was otherwise negative. No rickettsiae were seen in peritoneal smears.

Three gerbilles immune to the Calcutta strain were challenged with Karp strain inoculum and survived.

The 30 Karp-immune gerbilles were divided into 3 groups: 9 were inoculated with the Ceylon strain, 10 with the Calcutta strain, and 11 with Imphal strain infectious material. There were no deaths among these gerbilles.

The 9 gerbilles immune to the Imphal strain were divided into 3 groups of 3 which

were tested with the Ceylon, Calcutta, and Karp strains, respectively. All of the gerbilles survived.

The gerbilles which were tested with the Karp strain received 316,000 lethal doses; those tested with the Calcutta strain received 31,600 lethal doses; while those receiving Ceylon and Imphal infectious material survived 2,140 and 3,160 lethal doses, respectively. Thus, it is apparent that a solid immunity against heterologous strains of *R. orientalis* was present in these gerbilles. It is possible, of course, "interference phenomenon" may have exerted an effect of undetermined degree in these tests.

Summary. Gerbilles which were infected with tsutsugamushi disease and survived through treatment with PABA were tested 6 to 9 months later and found to be immune to homologous strains of *R. orientalis*. This is interpreted as evidence that the mode of action of para-aminobenzoic acid in scrub typhus is rickettsiostatic.

Cross-immunity tests revealed that these gerbilles were also immune to heterologous strains of *R. orientalis*.

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15288 P

Natural Occurrence of an "Induced" Antigen in Salmonella Cultures.*

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It is known¹⁻⁶ that H antigens of *Salmonella* can be markedly changed by cultivating the organisms in serum containing agglutinins for the flagellar components. Excepting *S. abortus-equi*⁴ and *S. paratyphi A*,⁵ all antigens

so obtained have had little or no relationship to naturally occurring H antigens of the genus, and have been referred to as induced

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¹ Kauffmann, F., *Z. Hyg.*, 1936, **119**, 104.

² Gnosspelius, A., *Z. Hyg.*, 1939, **121**, 529.

³ Kauffmann, F., and Tesdal, M., *Z. Hyg.*, 1937, **120**, 168.

⁴ Edwards, P. R., and Bruner, D. W., *J. Bact.*, 1939, **38**, 63.

⁵ Bruner, D. W., and Edwards, P. R., *J. Bact.*, 1941, **42**, 467.

⁶ Edwards, P. R., and Bruner, D. W., *J. Bact.*, 1942, **44**, 289.