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Further Report on Effect of Para-Aminobenzoic Acid in Experimental Tsutsugamushi Disease (Scrub Typhus).

E. S. MURRAY, C. J. D. ZARAFONETIS, AND J. C. SNYDER. (Introduced by S. Bayne-Jones.)

From the Cairo Unit of the United States of America Typhus Commission.

In a preliminary note experiments were described which indicated that the administration of the sodium salt of para-aminobenzoic acid (Na PAB) reduced the mortality of experimental tsutsugamushi disease (scrub typhus) in gerbilles.[1] Additional tests of Na PAB have now been completed with other strains of *Rickettsia orientalis*. The data of these further trials are presented below.

Description of the strains of R. orientalis. The sources of the Ceylon and Calcutta strains have been cited in another report.[2] The Karp strain[3] was obtained through the courtesy of the National Institute of Health, Bethesda, Maryland.* The inoculum was prepared from peritoneal fluid of infected gerbilles after one or more passages had been made in these rodents.

Method of administration of Na PAB in the diet. The plan of therapy as followed in Experiments 3 and 4 in the preliminary note, was changed slightly to improve the regularity of the food intake of the gerbilles. The changes were these: (1) water bottles were not used; (2) all of the food dishes were removed, cleaned, and filled with freshly prepared wet mash, and then replaced in the cages, every 8 hours during the 21 days of each test. The composition of the wet mash was essentially the same as previously described: 80 g of dried, powdered Egyptian bread, 2 g of sodium chloride, and 60 cc of milk constituted the basic mixture. For the controls 40 cc of tap water was added. For the treated gerbilles 40 cc of approximately 10.4% Na PAB solution was added. In most instances the food dishes were filled with slightly more of the mash than the gerbilles were able to consume in any 8-hour period. The control mash and the Na PAB mash were placed in the cages a few hours after the inoculation of *R. orientalis* suspensions in every test except Experiment 5. No attempt was made to determine the effect of delayed administration of Na PAB on the course of the infection.

Method of administration of Na PAB parenterally. All of the gerbilles received subcutaneous injections every 8 hours from the 5th or 6th to the 16th or 17th day after inoculation with *R. orientalis*. The control gerbilles received saline, approximately 0.1 cc per 25 g body weight. The treated gerbilles received solutions of Na PAB, approximately 0.1 cc per 25 g body weight, the solutions varied in concentration from 10.4 to 11.6% in the different tests. This plan of treatment, in which the oral intake was supplemented by parenteral therapy, is referred to hereafter as the "routine combined Na PAB therapy."

The Na PAB was prepared by the addition of 500 cc distilled water to 84 g of Na HCO_3 and 137 g of PABA. In some instances the resulting dark brown solution was evaporated to dryness at room temperature, and the powder thus obtained was weighed out for the preparation of the solution in distilled water. The concentrated solutions were adjusted to the desired strength by the addition

[1] Snyder, J. C., and Zarafonetis, C. J. D., Report to the Director of the U.S.A. Typhus Commission. Submitted for publication 9 October, 1944. In press.

[2] Zarafonetis, C. J. D., Report to the Director of the U.S.A. Typhus Commission. PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 113.

[3] McLimans, W. F., Grant, C. W., and Gersh, I., Report No. 1 (from the Naval Medical Research Institute, Bethesda, Maryland), 4 October, 1944.

* The authors wish to thank Lt. Comdr. A. Yeomans for transporting the infected animals to the Cairo laboratory.

conduction either of discrete excitations or of mass electrical currents were found to cause only a slight, almost negligible, interference with motor coordination.

The lesions consisted of intersecting transverse and longitudinal cuts at intervals of about 2.4 mm made subpially with a thin knife and extending roughly through the depth of the cortex to the underlying medulla. Because the cuts filled immediately with blood, the effect of the operation was to partition the cortex by numerous vertical walls of clotted blood of about 0.08 mm thickness. The cuts interrupted the horizontal transmission of excitations through the cortex itself, but left intact the axon interconnections looping downward through the white matter. These incisions were placed throughout the exposed surface of the cortical arm region including areas 6, 4s, 4, 1, 2, 5, and 7 as represented on architectonic charts with some overlap into the neighboring trunk, leg, and face areas. As a control of the effects of these incisions the corresponding cortical area was destroyed completely by excision in other animals.

The control animals showed a severe paralysis of the contralateral arm and hand which persisted with only slow improvement for a month or longer. In contrast partitioning the area with vertical incisions as described above caused practically no disruption of arm coordination. On the first day after operation no difference was noted in the use of the affected and normal arms in rapid running and climbing movements in a large cage. Even in fine movements of the fingers and hands in manipulating pieces of food, the difference between the normal and affected limbs was very slight. A little weakness in the manipulative finger movements of the affected hand and some preference in such movements for use of the normal hand were the only symptoms noted on the first day after operation. By the seventh day no trace of any motor deficit could be detected. Nor were any sensory or autonomic effects apparent although no special effort was made to test for these. Uninstructed observers could not, even with close study, determine at this time which was the affected arm. Thus although the electrostimulable points in the cortex for movement of shoulder, elbow, wrist, and digits had been separated from each other by incisions as described above, there was no incoordination in the arm and hand movements. The same results were obtained in three unilateral cases. The effects were similar also when lesions of the same type were placed in the opposite hemispheres of these three animals about three weeks after the first operations. Histological examination of the cortex showed that the knife cuts had clearly effected a vertical partitioning of the grey matter as intended.

The surprising lack of disturbance in motor coordination caused by putting multiple vertical partitions in the cortical arm field casts evident doubt on hypotheses of cerebral function which have assumed that horizontal intracortical interaction between and within cortical areas is extensive and an essential element of cortical integration. The experiments indicate that functional interaction between cortical loci is achieved almost entirely by the systems of axons passing through the white matter and that the intracortical interaction *per se* is largely vertical. This is in accord with the descriptions of intracortical synaptic relations presented by Lorente de Nó[1] in which he has pointed out that intracortical connections are established chiefly in the vertical direction with the cells of the various horizontal layers linked together in vertical chains.

Further work is under way to determine the effects of making the incisions increasingly numerous and closer together and also at increased depths into the white matter. Extension of the experiments to the visual cortex has been started.

[1] Lorente de Nó, Chap. XV in *Physiology of the Nervous System* by J. F. Fulton, Oxford University Press, N.Y., Inc., 1943.

combined Na PAB therapy." Titration of inoculum: dilutions in saline to $10^{-2.3}$, $10^{-3.6}$, and $10^{-4.9}$. One cc of each dilution inoculated into 4 gerbilles i.p. Results in Table II.

Experiment 10. Karp strain. Inoculum obtained from second passage in gerbilles of the material derived from white mice brought to Cairo from the National Institute of Health. Moribund gerbille sacrificed; 3 cc fluid from peritoneal cavity was lightly centrifuged for a few minutes; the supernatant constituted the "undiluted inoculum." This was diluted with saline to 10^{-1} and the latter was lightly centrifuged for a few minutes. Further tenfold dilutions were thereafter made without centrifuging. Broth cultures were negative. Treatment was "routine combined Na PAB therapy." All of the control gerbilles had characteristic autopsy findings. None of the 4 treated gerbilles which died had typical autopsy findings. Results in Table III.

Experiment 11. Karp strain. Inoculum obtained from second passage in gerbilles of the material derived from guinea pigs brought to Cairo from the National Institute of Health. Moribund gerbille sacrificed; 1 cc fluid obtained from peritoneal cavity; this was diluted with 3 cc saline and the resulting suspension was called "undiluted inoculum." Serial tenfold dilutions in saline were made. Broth cultures showed no growth. Treatment was "routine combined Na PAB therapy." All controls died with

TABLE III.
Experiment 10. Karp Strain.

	Controls		Treated	
Conc. of inoculum	Time of death of each gerbille, days after inoculation	Ratio survivors to total in group	Time of death of each gerbille days after inoculation	Ratio survivors to total in group
10^{-2}	8, 8, 9, 9, 10, 10, 10, 11, 12, 13	0/10		10/10
10^{-3}	11, 11, 11, 11, 11, 11, 12, 13, 13, 13	0/10	*11**	9/10
10^{-4}	12, 13, 14, 14, 14, 14, 15, 15, 15, 16	0/10	*16*†	8/9
10^{-5}	12, 15, 15, 17, 17	0/5	*13*,‡ *19*§	3/5
	Total	0/35	Total	30/34

The gerbilles weighed from 30 to 50 g at the time of inoculation; they were evenly distributed between control and treated groups according to their weights.

* Died in convulsion upon inoculation with R_x. Autopsy: chest filled with free blood; otherwise negative.

† Autopsy: minimal fluid in chest; no peritoneal exudate; liver and spleen only slightly enlarged.

‡ Autopsy: tremendous dilatation of gut; otherwise negative.

§ Autopsy: slight enlargement of liver and spleen; otherwise negative. The animals in this cage were sick for several days in the middle of the experiment, and the survivors improved.

TABLE IV.
Experiment 11. Karp Strain.

	Controls		Treated	
Conc. of inoculum	Time of death of each gerbille days after inoculation	Ratio survivors to total in group	Time of death of each gerbille, days after inoculation	Ratio survivors to total in group
10^{-2}	9, 9, 9, 9, 10	0/5	12, *15*	3/5
10^{-3}	9, 9, 9, 9, 10, 10, 10, 10, 10, 10, 10, 11, 11, 11	0/15	*13*, *18*, *19*	12/15
10^{-4}	14, 16, 16, 16, 16	0/5		5/5
10^{-5}	14, 14, 14, 14, 17	0/5		
	Total	0/30	Total	20/25

The gerbilles weighed from 30 to 50 g at the time of inoculation; they were evenly distributed between control and treated groups according to their weights.

The italicized figures in the treated gerbille column indicate that the animal did not show characteristic findings of *R. orientalis* infection at autopsy.

TABLE V.
Summary of Experiments 3,* 4,* 5, 6, 10, and 11, in Which Gerbilles Received "Routine Combined Na PAB Therapy."†

Conc. of inoculum	Controls		Na PAB treatment	
	Ratio survivors to total	% survivors	Ratio survivors to total	% survivors
"Undiluted"	0/8	0	2/8	25
10^{-1}	0/38	0	34/39	87
10^{-2}	0/31	0	25/30	83
10^{-3}	2/33	6	28/32	88
10^{-4}	0/15	0	13/14	93
10^{-5}	0/5	0	3/5	60
Total, all inocula	2/130	1.5	105/128	82

* The protocols of Experiments 3 and 4 were reported in the preliminary note.[1]
† The table does not include the control gerbilles which received inocula for titration if such inocula were not also given to corresponding groups of treated gerbilles.

characteristic autopsy findings. Four of the 5 treated gerbilles which died did not have typical autopsies. Results in Table IV.

In the 8 different trials of PABA in gerbilles infected with the Ceylon, Calcutta, Imphal and Karp strains of *R. orientalis* there were two routines of administration of PABA. The first routine was the feeding of a dry diet containing 3% of PABA in the acid form; parenteral therapy either was not given, or was inadequate to produce sustained concentration of PABA in the blood. There were no survivors in the 2 experiments of this type (1 and 2),[1] and merely a slight prolongation of survival time of treated gerbilles as compared with the controls. The blood concentration of PABA by this plan of therapy is usually low and frequently zero.

The second routine of therapy in our experiments was the combination of oral and parenteral administration of the highly soluble sodium salt of PABA. This routine assured a sustained concentration of PABA in the blood of the treated rodents during the entire period of the tests. The results of the experiments in which this routine was adhered to throughout (No. 3, 4, 5, 6, 10, and 11), are summarized in Table V.

The striking difference between the mortality of the controls and the treated gerbilles is clearly evident. There were 130 control gerbilles and of these 2 survived (1.5%). There were 128 treated gerbilles and 105 survived (82%). The data in Table V were obtained from the inoculation of doses of *R. orientalis* which ranged in severity from more than "one certainly fatal dose" to more than a "thousand certainly fatal doses."

There were 3 additional experiments (7, 8, and 9) with the Karp strain in which the conditions were not comparable to those in the experiments just reported. A change in the plan of therapy was followed in all or part of 7, 8, and 9, as a consequence of which the control as well as the treated gerbilles did not eat well during part of the tests and some were observed to be sick before such signs could be ascribed to infection with *R. orientalis*. In 2 of the 3 experiments the inoculum was derived directly from mice rather than from gerbille passage material. The results were less satisfactory than those in Table V. A summary of all gerbilles in Experiments 7, 8, and 9 shows that one control gerbille survived of 79 inoculated (1.3%). Eighteen treated gerbilles survived of 80 inoculated (23%).

The difference between the results with the "routine combined Na PAB therapy" and the results with other routines of therapy serves to emphasize the importance of the method of administration of the substance in any attempt to evaluate its effect on the course of the infection. This may account for the relatively slight effect of PABA on experimental tsutsugamushi disease in white mice reported by Peterson and Fox[4] and by

[4] Peterson, O. L., and Fox, J. P., Report to the Director of the U.S.A. Typhus Commission, 28 November, 1944.

Grant and McLimans.[5] In both of these reports PABA was given only by mouth, in the acid form. In the experiments of Grant and McLimans the concentration of PABA in the diet was roughly one-tenth the amount which proved successful in our experiments. Indeed, it is surprising that any effect was noted with such a small amount of drug.

It is our opinion that a thorough clinical trial of PABA against tsutsugamushi disease in humans is justified by the definite effect which PABA exerts on experimental tsutsugamushi disease in gerbilles. This favorable effect has been fully verified by repeated trials, using strains of *R. orientalis* from widely separated regions (India, Ceylon, New Guinea). Furthermore, the clinical evidence from PABA therapy of epidemic louse-borne typhus[6] has shown that it is safe to give PABA to man in large amounts.

Summary. Additional evidence has been cited to indicate that the sodium salt of p-aminobenzoic acid definitely reduced the mortality of experimental tsutsugamushi disease in gerbilles. A description has been given of the routine of administration of Na PAB which was successful. Data from other, less successful, routines have been reported to emphasize the importance of the mode of therapy in the evaluation of PABA in experimental infection with *R. orientalis*. A clinical trial of PABA in human tsutsugamushi disease is strongly recommended.

[5] Grant, C. W., and McLimans, W. F., Report No. 2 (from the Naval Medical Research Institute, Bethesda, Maryland), 11 November, 1944.

[6] Yeomans, A., Snyder, J. C., Murray, E. S., Zarafonetis, C. J. D., and Ecke, R. S., *J. A. M. A.*, 1944, **126**, 349.

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Certain Bacteriostatic Agents Added to Sera Used in Diagnostic Tests for Neurotropic Virus Infections.*

W. McD. HAMMON AND W. C. REEVES.

From the George Williams Hooper Foundation, University of California, San Francisco, Calif.

During the past five years a large variety of serological tests for virus infections, usually *in vivo*, but sometimes *in vitro*, have been made in this laboratory on several thousand serum specimens. Sera were generally tested for mouse protection against the viruses of both Western equine and St. Louis encephalitis and many were tested against the Lansing strain of poliomyelitis virus (adapted to the mouse by Armstrong[1]). Occasionally tests were made against the Eastern equine encephalomyelitis virus, the Japanese B encephalitis virus, a recently isolated strain of virus from California with neurotropic tendencies[2] and, more rarely, against still other neurotropic viruses. In addition to these tests, many of the specimens were subjected to experimental *in vitro* tests.

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[1] Armstrong, C., *Pub. Health Rep.*, 1939, **54**, 1719.

[2] Hammon, W. McD., and Reeves, W. C., unpublished data.