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Effects of Para-aminobenzoic Acid in Experimental Tsutsugamushi Disease (Scrub Typhus).

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Para-aminobenzoic acid (PABA) has been found to have a beneficial effect in experimental typhus[1,2] and in the treatment of classical louse-borne typhus fever in humans.[3] In the present report preliminary experiments are described which indicate that PABA may have a therapeutic effect against another rickettsial infection, experimental tsutsugamushi disease (scrub typhus).

The animals employed in these tests were *Gerbillus gerbillus* Olivier and *Gerbillus pyramidum* Geoffroy. Their susceptibility to tsutsugamushi disease has been described by Zarafonetis[4] who discussed the characteristics of the experimental infection produced by *R. orientalis* and cited the sources of the strains which were employed in the chemotherapy experiments presented below.

Four experiments are reported in this communication. In Experiments I and II, the "Imphal" strain was used. The inoculum was prepared from a moribund gerbille by washing the peritoneal cavity with 4 cc saline. One cc was injected intraperitoneally into young gerbilles (species *G. pyramidum*) which weighed 20 to 25 g. The control gerbilles were fed a diet consisting of dried, powdered Egyptian bread. For the treated group of gerbilles 3 g of powdered PABA were mixed with 97 g of dried bread. Water bottles were placed in the cages of both treated and control groups. The feeding of PABA was begun on the day of inoculation and was continued until all the animals were dead. In Experiment I no PABA was given parenterally. In experiment II one group of gerbilles received 10 mg of PABA subcutaneously twice daily, beginning on the 7th day after inoculation, approximately 36 hours before the average time of death of the control animals. Another group of gerbilles received no PABA either in the diet or subcutaneously until the seventh day. A few gerbilles were sacrificed for the measurement of blood concentrations of PABA; as expected, the blood levels fell to zero when the animals stopped eating with the onset of sickness.

The results of Experiment I and II are presented in Table I, which shows that there were no survivors in either experiment. There was a slight suggestion that the survival time was lengthened by the early addition of PABA to the diet. Subsequent tests showed that the inoculum probably contained between 100 and 1000 fatal doses.

In Experiments III and IV the "Ceylon" strain was used. The highly soluble sodium salt of PABA, referred to hereafter as NaPAB, was used instead of the relatively insoluble acid itself. The diet for the control gerbilles was a wet mash composed of 80 g of dried, powdered Egyptian bread, 2 to 4 g of sodium chloride, 60 cc of milk, and 40 cc of tap water. The diet for the treated gerbilles was identical except that 40 cc of 9% NaPAB were used instead of water in preparing the mash. Water bottles supplemented the diet of both treated and control groups. Feeding of the drug was begun immediately after inoculation and was continued until the experiments were arbitrarily terminated at the end of the 21st day. Since these rodents tend to eat only at night, the blood levels of PABA undoubtedly fluctuated widely, and it was decided to supplement the amount received in the diet by injec-

[1] Snyder, J. C., Maier, J., and Anderson, C. R., Report to the Division of Medical Sciences, National Research Council, Dec. 26, 1942.

[2] Hamilton, H. L., Plotz, H., and Smadel, J. E., Report to the Director of The United States of America Typhus Commission, 16 December, 1943. To be published.

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

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

TABLE I.

Data from Experiments I and II with the "Imphal" Strain of Tsutsugamushi Disease. Each Gerbille Was Inoculated Intraperitoneally with 1 cc of Undiluted Peritoneal Washings (Inoculum "A").

Exp.	Description of groups	No. of gerbilles in each group	Survival time of each gerbille, days	Avg survival time of entire group, days
I.	Controls	6	9, 9, 11, 11, 12, 12	10.7
	PABA (3% in diet begun zero day)	5	9, 10, 11, 16, 16	12.4
II.	Controls	6	7, 8, 8, 8, 9, 10	8.3
	PABA (3% in diet begun zero day; s.c. from 7th day on)	6	8, 8, 8, 9, 11, 12	9.3
	PABA first begun 7th day in diet and s.c.	6	8, 8, 8, 8, 9, 10	8.5

TABLE II.

Data from Experiments III and IV, with the "Ceylon" Strain of Tsutsugamushi Disease.

Exp. No.	Concentrations of inoculum	Controls: Time of death of each gerbille, days after inoculation	Controls: Ratio survivors to total in group	NaPAB treated: Time of death of each gerbille days after inoculation	NaPAB treated: Ratio survivors to total in group
III.	"A." Undiluted	6, 9, 9, 13	0/4	14, *15*, 18	1/4
	"B." Diluted 1/10	12, 12, 13, 14	0/4	*8*, *15*	2/4
	"C." " 1/100	11, 12, 13, 14	0/4	10*	3/4
	"D." " 1/1000	13, 18, 19	1/4	—	4/4
IV.	"A." Undiluted	9, 10, 10, 10	0/4	10, 12, *13*	1/4
	"B." Diluted 1/10	11, 11, 11	0/3	16	3/4
	"C." " 1/100	13, 13, 14, 15, 16	0/5	—	4/4
	"D." " 1/1000	20, 20, 20	1/4	—	3/3

* This gerbille was attacked by its cage mates on the 8th day, and a large area of skin was chewed away; it was isolated; at autopsy no exudate was found and the organs appeared to be normal; a very few rickettsiæ were seen in smears.

Where the day of death has been italicized, the autopsy findings were atypical, in that there were slight or no pleural and peritoneal exudates and the organs showed slight or no variation from normal in size and gross appearance. In 2 of these gerbilles, however, the liver was large and quite pale, a finding not previously encountered in tsutsugamushi disease in gerbilles.

TABLE III.

Summary of the Data from Experiments I, II, III, and IV Arranged to Show the Total Number of Gerbilles in the Control and the Treated Groups Which Received Different Concentrations of Infective Inoculum and the Percent Survival in Each Group.

Concentration of inoculum	Control gerbilles: Total No. in group	Control gerbilles: % survival	Treated gerbilles*: Total No. in group	Treated gerbilles*: % survival
"A" Undiluted	20	0	25	8
"B" Diluted 1/10	7	0	8	62½
"C" " 1/100	9	0	8	87½
"D" " 1/1000	8	25	7	100

* The dosage is described in the text.

tions of NaPAB at intervals of 8 hours. In Experiment III the parenteral administration was begun on the 7th day and continued until the end of the 16th day; the dose was 0.1 cc of 9% NaPAB. In Experiment IV the injections were started one day earlier and continued one day longer; the dose was increased to 0.15 cc of 9% NaPAB because the gerbilles were larger. In both experiments the controls were inoculated with 0.85% saline at the same intervals and in the same volumes as the treated gerbilles. No anesthesia was used. Titrations were made to determine the severity of the infecting dose of rickettsiæ. On 2 occa-

sions, blood PABA concentrations were measured 8 hours after a subcutaneous dose of 9 mg of NaPAB. The values were 3 and 5 mg per 100 cc, which were taken to indicate that the parenteral dosage schedule as supplemented by the amount taken in the diet probably was adequate to maintain a measurable concentration of PABA in the blood throughout the period of therapy.

In Experiment III most of the gerbilles were of the species *G. pyramidum,* though a few were *G. gerbillus*. The animals weighed less than 30 g at the beginning of the test. The inoculum consisted of peritoneal washings only. Table II shows that of a total of 16 gerbilles in each category, only 1 control gerbille survived, whereas 10 NaPAB treated gerbilles survived.

In Experiment IV all of the gerbilles were of the species *G. pyramidum*. The weight range was 30 to 40 g at the outset. The inoculum consisted of peritoneal washings in which brain and spleen of the same gerbille were suspended, so that the concentration of these organs in the "undiluted" inoculum was approximately 10%. It can be seen in Table II that only 1 control gerbille survived out of a total of 16 inoculated, whereas 11 of 15 NaPAB treated gerbilles survived.

The findings at autopsy in all of the control gerbilles were characteristic of experimental tsutsugamushi disease as previously reported, and rickettsiæ were demonstrated in smears of the peritoneal exudates. In the NaPAB treated groups, however, atypical findings were encountered as shown in the footnotes of Table II.

A summary of the 4 experiments has been prepared in Table III which shows the total number of animals and the percent survival in each group.

It seems clear from the data shown in Table III that the inoculum consisting of undiluted peritoneal washings (inoculum "A"), which contained roughly 100 to 1000 fatal doses, was so severe that the effect of PABA, or NaPAB, was overwhelmed; there were no survivors in the control groups (20 gerbilles) and only 2 survivors in the treated groups (25 gerbilles).

With inoculum "B," which contained roughly 10 to 100 fatal doses a definite difference was apparent. There were no survivors in the controls, as contrasted with 5 of 8 or 62½% in the NaPAB treated groups.

With inoculum "C," which contained between 1 and 10 fatal doses, the difference was even greater: none of the control gerbilles survived, as opposed to 7 of 8 or 87½% of the NaPAB treated gerbilles.

Inoculum "D," was not sufficiently concentrated to kill all of the control animals within the arbitrary 21-day limit. Two control gerbilles survived of a total of 8 inoculated. There were no deaths in the treated groups, however.

These results must be confirmed by tests using larger numbers of animals and other strains of *R. orientalis* before final conclusions can be drawn. The evidence thus far is highly suggestive, however, that the mortality from experimental tsutsugamushi disease can be reduced by the administration of the sodium salt of para-aminobenzoic acid.

Summary. The oral and parenteral administration of the sodium salt of para-aminobenzoic acid to gerbilles (*Gerbillus gerbillus* and *Gerbillus pyramidum*) experimentally infected with *R. orientalis* reduced the mortality. The results of these preliminary experiments indicate that this compound may have beneficial therapeutic action in the treatment of tsutsugamushi (scrub typhus) infection.

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