later the same sciatic nerve in each animal was inoculated as described above. Two of the animals died on the fourth day of undetermined cause, showing no signs of tetanus. The other six were sacrificed on the sixth day after inoculation. The toxin failed to reach the central nervous system because of the degeneration of the axis cylinder.

Another series of 8 guinea pigs was inoculated in like manner without freezing. Four of these received .5 cc. of tetanus antitoxin subcutaneously 10 minutes previous to the inoculation. The other four were given 0.1 cc. of the antitoxin into the sciatic nerve central to the cut end immediately after inoculation. All 8 of these animals were sacrificed 14 days later without any signs of tetanus. No attempt has been made to freeze the nerve and then allow for regeneration of the axis cylinder before inoculation.

The average length of the central stump of the sciatic nerve was 57 mm. The average time from inoculation until the opposite limb began to show tetany was 17 hours. Assuming that the tetanus toxin travels uniformly up the axis cylinder, the rate of progression then is 3.35 mm./hour. No attempt has been made to determine the method by which the toxin ascends the nerve fiber.

References

1. FRIEDEMANN, U. *Physiol. Rev.*, 1942, **22**, 125–145.
2. HOWE, H. A., and BODIAN, D. *Neural mechanisms in poliomyelitis*. New York: Oxford Univ. Press, 1942.

Therapy of Experimental Tsutsugamushi Disease (Scrub Typhus)[1]

LT. (JG) W. F. MCLIMANS, H (S), USNR, and LT. C. W. GRANT, H (S), USNR[2]

Naval Medical Research Institute, Bethesda, Maryland

Tsutsugamushi disease (scrub typhus), an acute febrile disease of the Asiatic areas, became of major military importance during the war in the Pacific theater. It was late in 1943 that the first strain of the etiological agent, *Rickettsia tsutsugamushi*[3] (Karp), was obtained by the U. S. Navy[4] for study in this country. At that time an investigation was begun to determine possible therapeutic agents that might be used in combating the experimental infection in Swiss mice.

It was observed during the course of these experiments that mice inoculated intraperitoneally with the Karp strain of *R. tsutsugamushi* and subjected to elevated oxygen tension, whether at sea level or increased pressure, showed a decreased mortality.

In a typical experiment 90 mice were inoculated intraperitoneally with 1,000 m.l.d. of the Karp strain. Immediately after inoculation, one group of 30 mice was placed in a standard oxygen tent and subjected to an oxygen tension of 50 per cent

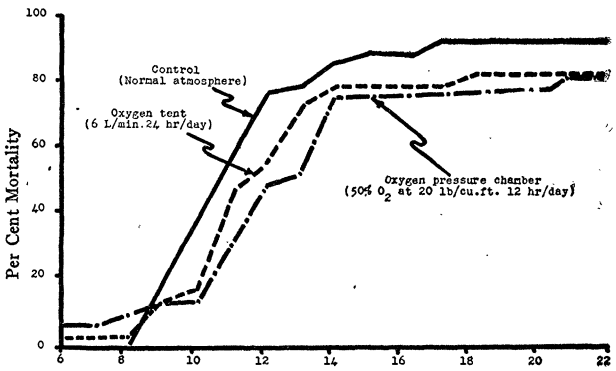

FIG. 1. Effect of oxygen tension upon the survival time and mortality of *R. tsutsugamushi* (Karp) infected mice.

maintained by a flow rate of 6 l./minute; a second group of 30 mice was placed in a pressure chamber containing 50 per cent oxygen in nitrogen at 20 pounds pressure; and a third group of 30 mice was kept under normal atmospheric conditions. It will be noted that increased oxygen tensions had a slight yet consistently beneficial effect on the survival time and the mortality of infected mice (Fig. 1).

On the basis of the action of this physiological factor it appeared advisable to test a series of agents for therapeutic activity under both environmental conditions—normal atmosphere and 50 per cent oxygen.

The institution of therapy was always delayed for at least 96 hours following inoculation of the mice. This period represented, as previously shown (*3*), the time usually required for the blood of mice inoculated with 1,000 m.l.d. of the Karp strain to become infective in sufficient titer to kill passage mice. It was thus assumed to be that period in the course of the infection which closely represented the end of the incubation period. Therefore, agents which proved to be efficacious when treatment was delayed for at least 96 hours could be classed as therapeutic rather than prophylactic.

The therapeutic agents were administered orally by mixing them intimately with the normal diet of ground fox chow.

One agent, methylthionine chloride (methylene blue), in contrast to the others, proved to be remarkably effective in combating the infection in Swiss mice under these conditions. It was observed that 0.2 per cent methylthionine chloride (MTC), added to the diet 96 hours following inoculation of the mice, reduced the mortality to 30–40 per cent, as contrasted to a 90–100 per cent mortality for the untreated controls. When oxygen therapy was instituted in conjunction with MTC, the mortality of the treated mice was further reduced to 20–30 per cent.

MTC has been used as a therapeutic agent in such diverse human infections as gonorrhea, tympanosomiasis, malaria, etc. (*1*). It has also been reported as an active agent in the

[1] The opinions or assertions contained herein are the authors' and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. This paper is based on a report of the authors entitled "Studies in tsutsugamushi disease. II: Experimental therapy of the infection in Swiss mice" (U. S. Naval Medical Research Institute, Research Project X-222, Rep. No. 2, November 9, 1944).

[2] Present addresses: Departments of Bacteriology, University of Minnesota and Brooklyn College, respectively.

[3] R. Lewthwaite has made a comprehensive survey of the literature regarding the correct nomenclature for the etiological agent of this disease and has recommended *R. tsutsugamushi*. Personal communication.

[4] This strain was isolated by F. M. Burnett, Royal Melbourne Hospital, Victoria, Australia.

therapy of experimental murine typhus infections in Swiss mice (*4*) and guinea pigs (*2*).

Since p-aminobenzoic acid (PABA) had previously been shown by Snyder, *et al.* (*5*) to be effective in the therapy of mice infected with the etiological agent of endemic typhus, an effort to determine the relative efficacy of MTC and PABA in

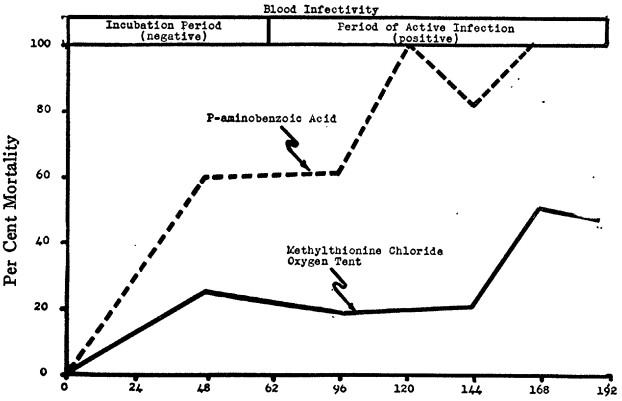

FIG. 2. Relative efficacy of MTC and PABA in *tsutsugamushi* (Karp) infected mice. Therapy interval indicates the elapsed time between inoculation of the mice and institution of therapy.

experimental *R. tsutsugamushi* infections was made. The concentrations of the two drugs in the ground fox chow were made equivalent—0.4 per cent. Institution of therapy was delayed for various time intervals up to and including 192 hours postinoculation. The results of this experiment (Fig. 2) show that while PABA was no longer effective when institution of therapy was delayed for more than 120 hours, MTC plus oxygen was still partially effective even when treatment was delayed up to 192 hours. Furthermore, the per cent mortality

TABLE 1

EFFECT OF MTC, MTC PLUS OXYGEN, AND PABA ON THE AMOUNT OF PERITONEAL FLUID FOUND ON AUTOPSY

Agent	No. of mice	Percentage of mice showing graded amounts of peritoneal fluid*				
		Neg.	+	++	+++	++++
MTC (normal atmosphere)....	56	39.2	44.7	13.6	0.0	2.3
MTC (oxygen).....	26	44.0	32.1	19.5	4.5	0.0
PABA.....	43	4.1	30.5	32.7	19.2	13.1
Control (untreated).....	44	9.1	15.9	45.4	15.9	13.6

* Peritoneal fluid graded as neg. = none; + = 0–0.2 cc.; ++ = 0.2–0.5 cc.; +++ = 0.5–1.0 cc.; ++++ = greater than 1 cc.

in all instances was significantly lower in the group treated with MTC.

When the concentration of PABA was increased to 1 per cent, its effectiveness as a therapeutic agent in the experimental infection increased but still was not of the order of magnitude of MTC.

Peritoneal fluid, one of the predominant gross pathological signs found at necropsy of untreated mice, was significantly reduced in quantity in the MTC-treated mice that died. In this respect, PABA-treated mice and the untreated control mice closely paralleled each other (Table 1).

In view of the well-established functions of MTC as an oxygen carrier and as a catalyst of numerous enzymatic reactions, studies on the mechanism of the action of this drug in the subject infection may reveal principles underlying the effective chemotherapy of virus and rickettsial diseases. Such studies are under way at this Institute.

These results clearly establish the beneficial effect of methylthionine chloride as a therapeutic agent in the treatment of *R. tsutsugamushi* infections in Swiss mice and the synergism of the drug with oxygen in such infections.

References

1. FISCHL, V., and SCHLOSSBERGER, H. *Handbook of chemotherapy.* Pt. I: *Metal free organic compounds.* Baltimore, Md.: H. G. Roebuck, 1933. P. 380.
2. KILKUTH, W., and SCHILLING, I. *Zbl. Bakt. Parasit. Infectionskr.*, 1944, **6**, 239–302.
3. MCLIMANS, W. F., and GRANT, C. W. A study of experimental tsutsugamushi disease in Swiss mice. U. S. Naval Medical Research Institute, Research Project X-222, Rep. No. 1, August 22, 1944.
4. OTTO, R., and SCHOFER, W. *Arb. Staatsinst. exp. Therap.*, 1936, **33**, 73–79.
5. SNYDER, J. C., MAIER, J., and ANDERSON, C. R. Report to Division of Medical Sciences, National Research Council, December 26, 1942.

Insecticidal Action of 1-Trichloro-2,2-Bis-(p-Bromophenyl) Ethane (Colorado 9)[1]

GEORGE M. LIST and MERLE G. PAYNE

Colorado Agricultural Experiment Station, Fort Collins

The compound made by condensing one molecule of chlorohydrate with two molecules of bromobenzene is 1-trichloro-2,2 bis(p-bromophenyl) ethane (*4*), which has been designated as Colorado 9. This has had a definite killing effect on certain insects and, like its relative, DDT, is stable and does not have an unpleasant odor.

Colorado 9 kills flies at the low spray level of 0.1 ml. of 5 per cent petroleum solution per cubic meter of air space. The flies lose their motor ability after 10 minutes and die within an hour. Early in 1945 it was determined by laboratory tests that Colorado 9 was lethal for the potato and tomato psyllid, *Paratrioza cockerelli* Sulc. During the growing season the material was used in potato plot tests by dissolving it in xylene at the rate of 1 pound to 1 quart of xylene and emulsifying by adding 1 part Triton 100 X to 50 parts of the above mixture. The spray solution carried 1 pound of Colorado 9 to 100 gallons of water. Five applications were made during the season, the usual schedule followed for psyllid control. At no time were any indications of plant injury from the spray applications detected. The variety of potato was the Irish Cobbler.

The yield of potatoes of marketable size ($1\frac{7}{8}$ inches in diameter or larger) has been found to be the most reliable measure of successful psyllid control. The treated plots yielded at the rate of 313.2 bushels/acre; untreated plots, at the rate of 22.8 bushels.

Colorado 9 was again used in plot tests in 1946 and at mid-

[1] Published with the approval of the director as paper No. 223, Scientific Journal Series, Colorado Agricultural Experiment Station.

This compound was synthesized in November 1944 by Merle G. Payne and E. L. Bailes. The latter, at that time a member of the chemistry section of the Colorado Experiment Station, has since resigned.