streptomycin, under the conditions of these investigations, was not readily absorbed through the intestines.

(4) The *in vitro* streptomycin resistance of cultures, isolated during therapy, did not differ from cultures isolated from blood and stools prior to the administration of streptomycin.

(5) The contrast between the very large dosages coupled with blood and fecal isolations during therapy, and the comparatively low *in vitro* resistance of these cultures, indicated the possible presence in the body of a substance inhibitory to streptomycin.

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PARA-AMINOBENZOIC ACID—ITS EFFECTIVENESS IN SPOTTED FEVER IN GUINEA PIGS[1]

IN contrast with the spectacular successes achieved by chemotherapy in bacterial infections, chemotherapy in viral and rickettsial diseases is in general noneffective. This applies particularly to the sulfonamide agents. Greiff and Pinkerton[2] have shown that sulfanilamide does not inhibit rickettsial growth in egg yolk-sac. Still more striking are the observations by Snyder, Maier and Anderson[3] that the sulfonamide drugs have a deleterious effect in experimental typhus. These phenomena, which offer another evidence of the apparently different metabolic requirements and activities of the intracellular rickettsiae, led Greiff, Pinkerton and Moragues[4] to the use of "enzyme activators" in rickettsial infections. The inhibitory action of p-aminobenzoic acid (PABA) on rickettsial growth was observed in egg yolk-sacs infected with murine typhus rickettsiae. A favorable effect of this drug on murine typhus infection in mice was also reported.[4] The beneficial effect of PABA on the clinical course of louse-borne typhus in man was established by the United States of America Typhus Commission unit in Egypt.[5]

On the basis of these observations PABA was tested by us in guinea pigs infected with Rocky Mountain spotted fever. The strain of tick origin (Rocky Mountain Laboratory) proved highly virulent to guinea pigs: the regular 72 hours' incubation period is followed invariably by high fever of 5 to 8 days' duration. The death-rate in infected guinea pigs is 100 per cent.

The present work was directed into the possible protection of animals against the disease by the use of PABA. For this purpose guinea pigs were given the drug in proportion of 2 gm of the powder per each 100 gm of high protein feed *ad lib.* The administration of this mixture was started shortly after the intra-abdominal injection of the infective material (spleen or blood). In other series the drug was given 24 hours prior to the infection or 24, 48 and 72 hours thereafter. The daily PABA treatment was then continued for 7 to 10 days, after which time it was withdrawn.

As compared with the clear-cut febrile and fatal disease in control animals a striking change in the response of test guinea pigs was observed. Out of 12 guinea pigs in which PABA intake preceded the injection by 24 hours three animals showed fevers of 1 to 3 days' duration; two guinea pigs reacted with slight and late elevations for 1–2 days, while seven animals remained completely afebrile. Two deaths were recorded. In another series of 17 guinea pigs treated with PABA beginning with the day of spotted fever injection, eleven animals remained afebrile during two weeks' observation, three reacted with one or two days' fever, two developed typical spotted fever and died, while one died showing no fever. As a whole, in 80 per cent. of test guinea pigs the fever was either entirely absent or reduced to abortive mild attacks.

When PABA was administered during the incubation period 30 per cent. of guinea pigs remained afebrile even when the drug was given 72 hours after injection. However, the animals of this series showed a rather erratic appetite, resulting in an inefficient intake of PABA.

These facts indicate that PABA, given before or shortly after infection, can prevent the appearance of clinical manifestations of the fatal spotted fever. In the large majority of test animals the visible symptoms (fever and scrotal reaction) were entirely absent. Nevertheless, some afebrile guinea pigs showed pathological lesions (splenomegaly, pneumonitis) typical for spotted fever. When the spleen was injected into normal guinea pigs the latter reacted with typical spotted fever like the controls. The surviving afebrile animals were then reinoculated with spotted fever rickettsiae and showed complete immunity. It seems, therefore, that the absence or mildness of clinical symptoms indicate rather a suppressive than destructive effect of p-aminobenzoic acid on spotted fever rickettsaie with the result of

[1] Study assisted by a grant from the American Foundation of Tropical Medicine, Incorporated.

[2] D. Greiff and H. Pinkerton, *Proc. Soc. Exp. Biol. and Med.*, 55: 116–119, 1944.

[3] J. C. Snyder, J. Maier and C. R. Anderson, Report to the Division of Med. Sciences, Nat. Res. Counc., December 26, 1942.

[4] D. Greiff, H. Pinkerton and V. Moragues, *Jour. Exp. Med.*, 80: 561–574, 1944.

[5] A. Yeomans, J. C. Snyder, E. S. Murray, C. J. D. Zarafonetis and R. S. Ecke, *Jour. Am. Med. Asn.*, 126: 349–356, 1944.

inapparent (subclinical) infection. This phase of biochemical activity is associated with or followed by immune phenomena by which the guinea pig acquires active resistance to subsequent infection.

The present study still in progress extends the previously observed effectiveness of p-aminobenzoic acid in other, milder rickettsioses[4, 5] and proves its protective value in the highly virulent spotted fever.

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STUDIES ON THE MECHANISM OF ANTIBACTERIAL ACTION OF 2-METHYL-1,4-NAPHTHOQUINONE[1]

THE antibacterial activity of quinones has been recognized since 1911.[2, 3, 4, 5, 6, 7] Interest in this phase of their activity has been stimulated in recent years by the discovery of the quinone structure in antibiotics derived from molds.[7, 8] Synthetic 2-methyl-1,4-naphthoquinone as tested in this laboratory has been shown to be bacteriostatic and bactericidal for both gram-positive cocci and gram-negative bacilli, and a similar effect has been noted on many species of fungi. It was found that minimum effective bacteriostatic and bactericidal concentrations for *Escherichia coli* in a chemically defined medium were lower than in the usual peptone broth or agar (0.00125 per cent. to 0.0025 per cent. instead of 0.005 per cent. to 0.02 per cent.); also that in Brewer's thioglycolate medium, both bacteriostatic and bactericidal activity of concentrations as high as 0.04 per cent. were nullified in freshly prepared medium, although in medium prepared some three months previously and thoroughly shaken immediately before use, minimum effective bacteriostatic and bactericidal concentrations were approximately the same as in other peptone media. The effect of the fresh Brewer's medium did not appear to be due to its anaerobic properties, since tests on agar plates incubated in an anaerobic jar gave the same endpoints as those obtained on aerobic incubation. The following work was undertaken in an attempt to explain this behavior.

METHODS

The medium used was McCleod's synthetic medium,[9] with asparagin and $(NH_4)_2SO_4$ as sources of nitrogen, glucose, NaCl, phosphates and traces of Fe, Ca and Mg, dispensed in 8″ by 1″ test-tubes in accurately measured amounts.

The quinones employed included 2-methyl-1,4-naphthoquinone; the water-soluble sodium bisulfite addition product of 2-methyl-1,4-naphthoquinone; 2-methyl-3-methoxy-1,4-naphthoquinone, 2-methyl-3-chloro-1,4-naphthoquinone and 2,6-dimethyl-1,4-naphthoquinone, all pure synthetic compounds prepared in the chemistry research laboratories of this corporation. Solutions were made up aseptically in acetone or water, depending on their solubilities, in concentrations 25 times as strong as the highest final concentration desired in the medium. Serial dilutions were made by halves and were added in 1 ml amounts to 24 ml of sterilized medium (to 23 ml if a substance to be tested for antagonism of antibacterial action was also to be added). Substances to be tested for antagonism of antibacterial action were prepared in sterile aqueous solution and 1 ml of a standard dilution was added to each culture tube of a series containing varying concentrations of the quinone. Acetone and water controls were included in each series. The inoculum of *Escherichia coli* was 0.1 ml of a 24-hour culture in the synthetic medium diluted 1:4 (approximately 20 to 25 million bacteria, or 800,000 to 1 million per ml of culture). Incubation was at 37° C. except when the volatile ethyl mercaptan was used, when both mercaptan and quinone control series were incubated at 30° C. Readings for bacteriostasis were made daily for 4 to 5 days by observations of visual turbidity. Daily subcultures were made by streaking each culture on an agar plate to determine whether organisms were killed or merely kept from growing.

Nitroprusside tests for sulfhydryl groups were made on mixtures of solutions of quinones and (a) thioglycolic acid neutralized with sodium carbonate and (b) cysteine hydrochloride by adding 3 N NH_3 and a dilute solution of sodium nitroprusside.

RESULTS

Thioglycolic acid neutralized with sodium carbonate and sodium thioglycolate (Eastman) in molar concentrations greater than 2-methyl-1,4-naphthoquinone suppressed both bacteriostatic and bactericidal activity of the quinone on *E. coli* in synthetic medium. This effect might have been due either to their action as reducing agents or to the sulfhydryl groups; consequently other reducing agents were tested. Sodium bisulfite and sodium hydrosulfite partially antagonized

[1] A preliminary report.
[2] W. Thalhimer and B. Palmer, *Jour. Infect. Dis.*, 9: 181, 1911.
[3] E. A. Cooper, *Biochem. Jour.*, 6: 362, 1912.
[4] G. T. Morgan and E. A. Cooper, *Biochem. Jour.*, 15: 587, 1921.
[5] G. T. Morgan and E. A. Cooper, *Soc. Chem. and Ind.*, 43: 352, 1924.
[6] W. D. Armstrong, W. W. Spink and J. Kahnke, *Proc. Soc. Exp. Biol. and Med.*, 53: 230, 1943.
[7] A. E. Oxford and H. Raistrick, *Chem. and Ind.*, 61: 128, 1942.
[8] S. A. Waksman and H. B. Woodruff, *Jour. Bact.*, 44: 373, 1942.
[9] Colin McCleod, *Jour. Exp. Med.*, 72: 217, 1940.