SPECIAL ARTICLES

THE INFLUENCE OF METHYL CHALCONE OF HESPERIDIN ON THE TOXICITY OF MAPHARSEN IN RABBITS[1]

THE rapid treatment of early syphilis has attracted wide interest because of the benefit to the patient and the community from the completion of treatment in a matter of days. The toxicity of the methods used so far has been too high, however, to warrant their routine adoption.

The most serious toxic manifestation has been arsenical encephalopathy which, from published reports, has occurred in about 1.3 per cent. of patients given treatment with mapharsen.[2,3,4] The mortality has been about 0.3 per cent. The clinical findings range from headache and dizziness to convulsions and death. Elevation of the protein in the spinal fluid is regularly present. The pathological findings in man vary greatly. Hemorrhage may or may not be present, but by far the commonest finding is capillary damage. Goldstein and Stevenson[5] observed similar lesions in the brains of rabbits treated intensively with toxic doses of mapharsen. These findings suggest that the chief pathological alteration in arsenical encephalopathy is an increase in capillary permeability.

Beginning in 1940, we performed experiments in rabbits with numerous agents in an effort to prevent cerebral reactions from mapharsen, but none of these were successful. In 1936 Szent-Györgyi and his co-workers[6] suggested the existence of an anticapillary permeability factor which they called vitamin P, but the existence of this factor has not been definitely established. Goldfarb[7] employed an aqueous extract prepared from the whole lemon in the treatment of psoriasis, and suggested its use as a possible preventive of arsenical encephalopathy in patients receiving intensive treatment with mapharsen. As the result of this suggestion it was considered desirable to conduct preliminary experiments in rabbits with the lemon juice extract.

Lemon juice extract was fed to 18 New Zealand white male rabbits, each weighing approximately 2 kilograms, as follows: 10 cc of extract was administered by stomach tube once daily for two weeks prior to and during the administration of mapharsen. Mapharsen was injected intravenously twice daily for four days in doses of 8 milligrams per kilogram. Ten animals received mapharsen alone in an identical manner and served as controls. Survival rates in the two groups were: mapharsen and lemon juice, 28 per cent.; mapharsen alone, 10 per cent. This difference was not statistically significant.

Wawra and Webb[8] reported the isolation of a chalcone of hesperidin from lemon peel, which, in their preliminary experiments, appeared to decrease the fragility of capillaries and to prevent localized hemorrhages. Later a methyl chalcone was developed by the Research Department of the California Fruit Growers Exchange in order to prevent reversion of the chalcone to the closed ring of hesperidin. This product was made available to us, and three experiments were performed in which a total of 30 rabbits received methyl chalcone and mapharsen, while 30 received mapharsen alone. Methyl chalcone was injected intravenously in doses of 10 or 30 milligrams per kilogram once daily for 7 days prior to the administration of mapharsen and for the 4 days of mapharsen administration. Mapharsen was injected intravenously twice daily for 4 days in doses of 8 milligrams per kilogram. The results are shown in Table I. Survival rates, determined after three weeks of observation following the last treatment, were as follows: mapharsen and methyl chalcone, 90 per cent., mapharsen alone, 57 per cent. The difference in survival rate between the two groups is statistically significant, the relative deviate being 2.9.

TABLE I

Exper. No.	Methyl chalcone mg/kg per dose	Mapharsen + methyl chalcone: Number of animals: Total	Died	Survived	Per cent. survival	Mepharsen alone: Number of animals: Total	Died	Survived	Per cent. survival
1	10	5	1	4	80	5	3	2	40
2	30	15	1	14	93	10	4	6	60
3	30	10	1	9	90	15	6	9	60
Total		30	3	27	90	30	13	17	57

The influence of methyl chalcone of hesperidin upon the spirocheticidal effect of mapharsen was studied *in vitro*. Exudate containing abundant *Treponema pallidum* was obtained from patients with mucous membrane lesions of secondary syphilis. Dark field preparations were made by mixing equal volumes of the exudate and the solution to be tested by means

[1] Supported in part by funds from the following agencies: The New York State Health Department, the Louis Livingston Seaman Fund, the California Fruit Growers Exchange Research Fund of New York University College of Medicine.

[2] L. Chargin, *Arch. Dermat. and Syph.*, 42: 248, 1940.

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[4] E. W. Thomas, Gertrude Wexler and B. Dattner, *Am. Jour. Syph., Gon. and Ven. Dis.*, 26: 529, 1942.

[5] D. H. Goldstein and L. D. Stevenson. To be published.

[6] A. Bentsath, S. Rusznyak and A. Szent-Györgyi, *Nature*, 138: 798, 1936. L. Armentano, A. Bentsath, T. Beres, S. Rusznyak and A. Szent-Györgyi, *Deutsche med. Wchnschr.*, 62: 1325, 1936.

[7] A. E. Goldfarb, *Arch. Dermat. and Syph.*, 43: 536, 1941.

[8] C. Z. Warwa and J. L. Webb, SCIENCE, 96: 302, 1942.

of calibrated red blood cell pipettes. The following preparations were set-up:

(1) Saline + spirochete fluid.
(2) Chalcone 1: 1,333 + spirochete fluid.
(3) Chalcone 1: 1,333 + mapharsen 1: 4,000 + spirochete fluid.
(4) Mapharsen 1: 4000 + spirochete fluid.
(5) Mapharsen 1: 4000 + glutathione 1: 400 + spirochete fluid.
(6) Glutathione 1: 400 + chalcone 1: 1,333 + spirochete fluid.
(7) Glutathione 1: 400 + spirochete fluid.

All dilutions are final dilutions. All reagents were adjusted to pH 7 ±.

The results of six experiments are shown in the

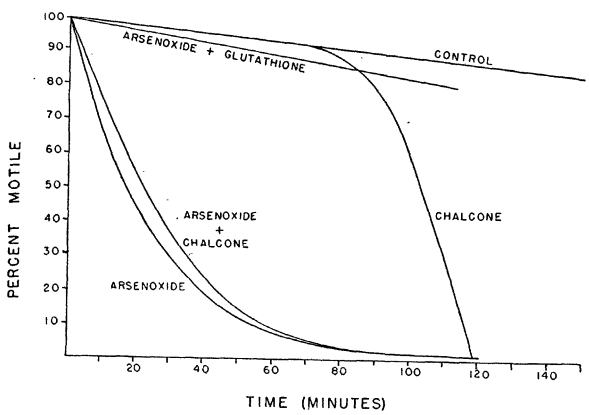

FIG. 1. Effect of various agents and their combinations on the motility of *Treponema pallidum in vitro*.

graph. The curves represent the average percentage of spirochetes retaining motility at the stated intervals. In the concentrations used, methyl chalcone did not inhibit the spirocheticidal action of mapharsen *in vitro*. Methyl chalcone appeared to exert independent spirocheticidal activity between 90 and 120 minutes after mixture with spirochetes. This effect could be abolished by glutathione.

These preliminary observations indicate that the methyl chalcone of hesperidin may be capable of diminishing the toxic effects of mapharsen without inhibiting its spirocheticidal effect, and that it may possess independent spirocheticidal activity to a mild degree. Further studies are in progress to explore these possibilities.

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THE ANTIBACTERIAL EFFECT OF ENZYMATIC XANTHINE OXIDATION[1]

RAISTRICK and his colleagues[2] made the curious observation that an oxidase may display antibacterial activity. They had observed a bactericidal activity with certain protein fractions from *Penicillium notatum* which definitely was not attributable to the ether-soluble, low-molecular penicillin. Eventually this bactericidal effect was traced to glucose oxidase, a flavoprotein which catalyzes the reaction:

$$\text{glucose} + O_2 \longrightarrow \text{gluconic acid} + H_2O_2$$

The generated hydrogen peroxide was thought to be mainly, if not exclusively, responsible for the bactericidal effect of the enzymatic system.[3] The name, notatin, was suggested for this enzymatic bactericide. Simultaneously two similar reports appeared: bactericidal protein-fractions derived from *Penicillium notatum* were described by Kocholaty[4] under the name of penatin and by Doisy *et al.*[5] under the name penicillin B.

Glucose oxidase, although not in its present role, has been known for some time. It is an enzyme found commonly in molds[6] and most abundantly in *Aspergillus niger*. It was recognized as a flavin enzyme.[7] Judging from the latest communications by Doisy *et al.*,[8] Kocholaty,[9] and by Birkinshaw and Raistrick,[3] there seems to be no reasonable doubt that the common mold glucose oxidase is the active principle in penatin and penicillin B as well as in notatin.

The conclusion reached by Raistrick *et al.* that their antibacterial activity was due essentially to the generation of hydrogen peroxide invited the rather obvious deduction that other oxidases may be found which act similarly. To prove this, it was decided to carry out appropriate experiments with xanthine oxidase. This H_2O_2 generating[10] enzyme was chosen because it may be easily obtained relatively free from counteracting factors, such as catalase, peroxidase, etc. Furthermore, the natural occurrence of this enzyme in milk suggested that with its appropriate substrate it may

1 Aided by a grant from the Commonwealth Fund.

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