

derived either from the original tobacco-plant material or from certain bacteria which were isolated from the solution of virus before filtration through the collodion filter. Accordingly, we suggest that great caution must be observed in interpreting the significance of analyses of materials derived from living tissues when subjected to delicate procedures of assay.

Conclusions. The presence of riboflavin, pantothenic acid, and biotin could not be demonstrated in significant amounts in moderately concentrated solutions of purified tobacco-mosaic virus.

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Sulfanilylguanidine in Control of Salmonella Infection and Carrier State in Mice.

PARKER R. BEAMER. (Introduced by Robert A. Moore.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

The presence of an enzootic Salmonella infection in our laboratory colonies of mice has prompted an investigation into the etiology, control, and nature of this disease. Data gathered from animal autopsies and bacteriological studies indicate that in 2 strains the infection follows different courses. The 2 strains of mice, Akh and Rfi, were developed by Furth of Cornell University Medical School for his studies of leukemia. Seventy to 80% of the Akh animals die of lymphoid leukemia if they live to be over 8 months of age. The Rfi mice carry myeloid leukemia and 1% die of the disease. Bacteriological studies of stool cultures revealed a significantly higher incidence of infection in Rfi mice than in Akh animals. Autopsies performed on 26 Akh mice in a 2-months' period revealed gross evidence of Salmonella infection in only 2, whereas gross evidence of this disease was seen in 17 animals in a series of 35 Rfi mice autopsied during the same period. The typical lesions seen at autopsy were numerous, irregularly distributed, white areas of focal necrosis, variable in

shape, 0.5 to 5 mm in diameter, occurring most frequently in the liver and spleen. Small hemorrhagic areas and minute ulcerations were occasionally present in the intestinal tract. In every instance where attempted, an organism identified as *Salmonella enteritidis* was isolated from the lesions in both strains of mice. On the basis of preliminary studies, to be completed and reported later, the organisms isolated from both strains of mice appeared to be identical.

Cultures from individual animals were made as follows: Fecal pellets were collected directly from the animals on sterile swabs and emulsified in 2 cc of sterile tryptose broth before streaking on brilliant green lactose agar plates¹ (extract agar (pH 7.0), 1% lactose, 0.1% dextrose, 1% Andrades indicator, brilliant green 1-250,000). Plates were incubated at 37°C and observed at intervals for 48 hours. Typical organisms were identified by fermentation reactions in several carbohydrate media and confirmatory microscopic agglutination tests were performed. The mice were kept in enamel pans with wire covers. The food was suspended in a wire container and water was supplied in an 8 oz bottle. The water bottles were sterilized once a week. The bedding was wood-shavings and the pans were cleaned every 4 to 7 days.

The incidence of infection in the colony as a whole was based on one plate culture. This is not as efficient as a series of cultures, since results of this investigation and others reported in the literature show that, although many mice are continuous excretors of the organisms, others excrete the organisms intermittently. Thirty-five pairs of Akh breeding mice showed an incidence of 31% (males 26%, females 37%). There were 18 pans in which both mice were negative, 12 in which only 1 was positive, and 5 where both mice were positive. Further study with a series of stool cultures for each animal indicated the above figures should be higher, since some of the mice were intermittent excretors. Eight pairs of Rfi breeding mice were available for examination; 13 (81%) of these were positive. The incidence in males and females was not notably different. Among the stock mice, 3 to 10 males or females per cage, the incidence in Akh mice was 14% compared to 50% in Rfi mice.

On the basis of the reports by Marshall and his associates^{2, 3} of beneficial results from the use of sulfanilylguanidine in the treatment

¹ Buchbinder, L., Hall, L., Wilens, S. L., and Slanetz, C. A., *Am. J. Hyg.*, 1935, **22**, 199.

² Marshall, E. K., Jr., Bratton, A. C., White, K. H., and Litchfield, J. T., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.

³ Marshall, E. K., Jr., Bratton, A. C., Edwards, L. B., and Walker, E., *Bull. Johns Hopkins Hosp.*, 1941, **68**, 94.

of children with bacillary dysentery, it seemed desirable to investigate the effect of this drug in mice infected with *Salmonella enteritidis*. Several pairs of Akh breeding mice were cultured serially at 3- to 6-day intervals and 16 pairs showing at least 2 positive plates out of 4 were selected for study. The drug, which is relatively insoluble, was suspended in water so that 0.1 cc contained the desired dose. An initial dose of 12-15 mg was given to one mouse in each cage followed by daily doses of 6-8 mg. Stool specimens collected during the period of chemotherapy and thereafter were cultured on brilliant green lactose agar to which p-amino benzoic acid was added in a final concentration of 10 mg %. During the first 5 days of treatment duplicate plate cultures from 12 of 15 treated mice were negative for Salmonella organisms. Seven to 10 days after starting chemotherapy 2 of the above 12 contained a few Salmonella colonies. The dose of sulfanilylguanidine was then increased to 13 mg and finally to 20 mg daily. These larger doses were given in powder form directly into the pharynx, followed by a few drops of water. After a period of 17 days, during which the stools were cultured in duplicate 4 times on brilliant green lactose agar with p-amino benzoic acid added, 11 of the 15 treated mice showed consistently negative cultures while the incidence of positive stools was unchanged in the untreated mates. Three of the treated mice had consistently positive stools and an additional 1 had only 2 to 6 Salmonella colonies per plate. All the treated mice were isolated into individual cages and the drug was discontinued after 2 days. During the next 70 days frequent stool cultures were made and in only 2 of the negative animals did the stool cultures become positive for Salmonella organisms.

Since the drug appeared to have some beneficial effects, we began treatment of Rfi stock mice by dissolving 150 mg of sulfanilylguanidine in 100 cc of drinking water. On the basis of measured water consumption, it was calculated that each mouse received 6 or more mg of drug daily. A series of 3 stool cultures were made in duplicate at the beginning of the experiment and repeated after 39 days of treatment. If one arbitrarily classifies the mice as +, ++, and +++ on the basis of the number of positive stool cultures in the series of 3, there were: (1) 17 +++ animals treated, 2 of which became negative, 7+, 5 ++, and 3 remained +++; (2) 18 ++ animals treated, 7 becoming negative, 7 +, 3 remaining ++, and 1 changing to +++; (3) 6 + animals treated, 4 of which turned negative and 2 became +++. Thus, it is seen that in most instances the drug appeared to have a beneficial effect, but in 3 mice the incidence of positive plates was increased.

The results obtained in this study indicate that *Salmonella* infection in laboratory colonies of mice may be effectively reduced if carriers are isolated and treated with chemotherapy. In our experiments, treatment of infected animals with sulfanilylguanidine resulted in a decrease of *Salmonella* organisms in stool cultures. In those few instances where chemotherapy is not effective the mice may be sacrificed in order to decrease the sources of infection.

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Pigment Observed in Cultures of Hemolytic Streptococci Belonging to Lancefield Group A.*

S. M. WHEELER AND G. E. FOLEY. (Introduced by J. F. Enders.)
From the Department of Preventive Medicine, Harvard Medical School, and House of the Good Samaritan, Boston, Mass.

Pigment production is not often observed in the growth of the hemolytic streptococcus. Durand and Giraud,¹ in a study of 124 cases of human streptococcus infection, reported the isolation of pigmented strains in 11 instances. Olivieri² reported the isolation of pigmented hemolytic streptococci from chronic pyelonephritis. Since these studies were made before the development of serological grouping, it is impossible to interpret the observations in terms of the newer classification. More recently, pigment has been observed in strains belonging to Lancefield's Group B, and occasionally to Lancefield's Group D.³ As far as could be determined from the literature, the phenomenon of pigment production has not been described in strains belonging to Group A.

During an epidemiological study of hemolytic streptococcus infections involving several thousand cultures, pigmented colonies were observed on several occasions in the cultures of 3 patients. A fourth pigmented strain was observed from a case of scarlet fever in an Army camp. The data relating to these strains are summarized in Table I. All strains were classified as to group by the precipitation method of Lancefield,⁴ and serological type was determined by

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¹ Durand, T., and Giraud, P., *Compt. rendu. Acad. d. sc.*, 1923, **177**, 1333.

² Olivieri, J., *J. d'urol.*, 1929, **27**, 484.

³ Lancefield, R. C., *J. Exp. Med.*, 1934, **59**, 459.

⁴ Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.