calcium pantothenate further improve the filtrate factor deficiency picture (Table).

Summary. Calcium pantothenate stimulates growth and prevents pattern graying (but not stippling) in filtrate factor deficient rats. The requirement for growth is less than for the prevention of pattern graying. Black rats receiving Ca pantothenate either prophylactically or curatively have stippled fur.

A liver filtrate stimulates growth to a greater extent than does Ca pantothenate and completely protects against graying. It would thus appear that pantothenic acid does not completely replace liver filtrate.

A concentrate prepared by the continuous extraction of cane molasses with ether both stimulated growth and prevented graying while a chloroform extract prepared from it was without activity.

Inositol added to Ca pantothenate does not significantly improve the results obtained with calcium pantothenate alone.

Rats maintained on a 24% casein diet apparently have no need for choline.

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Course and Chemotherapy of Experimental Relapsing Fever in the Chinese Hamster (*Cricetulus griseus.*)

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Many attempts have been made to develop in laboratory animals experimental relapsing fever of sufficient intensity and duration to permit immunologic and therapeutic studies. While monkeys and rodents are susceptible, different strains of relapsing fever spirochetes cause considerable variation in the reaction of the host. Most spirochetes produce infections of short duration in rats, mice and guinea pigs. One exception is *S. sogdianum,* a rare spirochete, which produces in guinea pigs a disease characterized by 2 to 4 relapses.[1]

While the treatment of relapsing fever usually is successful with the arsphenamines many strains of spirochetes have not responded

[1] Sautet, J., *Marseille-Méd.*, 1937, **74**, 273; from *Trop. Dis. Bull.*, 1938, **35**, 499.

satisfactorily.[2-5] Moreover, spirochetes may disappear from the peripheral blood and remain in the brain and other tissues after therapy. Buschke and Kroó[6] first emphasized the occurrence of residual infection and their observations have been confirmed by others.

Since the chemotherapy of experimental relapsing fever cannot be studied properly unless the course of infection permits adequate treatment and follow-up an attempt was made to develop a suitable infection in a small laboratory animal. We were fortunate in obtaining such an infection with ease in the Chinese hamster *(Cricetulus griseus)* by a California strain of spirochetes transmitted by *Ornithodorus hermsi*—Wheeler.* This strain is more satisfactory than others in Chinese hamsters since the disease is characterized by typical relapses in these animals. Intraperitoneal inoculations of blood from infected mice or hamsters into stock hamsters were invariably successful when 0.15 or 0.2 cc of a dilution of 1 part of tail blood in 9 parts of an aqueous solution containing 0.9% sodium chloride and 2% sodium citrate were used. The infection can be maintained without difficulty in hamsters.

When inoculations were made from animals of one species to those of another the course of infection differed from that observed in animals infected by others of the same species. In 20 mice inoculated with mouse blood the incubation period varied from 22 to 72 hours. Infections were most intense from the second to the fifth day and then gradually decreased with no definite relapses. This is in agreement with Beck's findings.[7]

In 53 hamsters the incubation period varied from 24 to 72 hours. When inoculations were made with mouse blood the incubation period was somewhat shorter, and spirochetes appeared in the peripheral blood in from 24 to 48 hours. There were 3 or 4 attacks characterized by spirochetemia each of from 4 to 6 days' duration with intervals usually of 24 hours. The intensity of infection was

[2] Kritschewski, J. L., *Klin. Woch.*, 1927, **6**, 441; from *Trop. Dis. Bull.*, 1927, **24**, 689.

[3] Dickinson, P. S., *Z. f. Hyg. u. Infektionskr.*, 1932, **113**, 683; from *Trop. Dis. Bull.*, 1932, **29**, 564.

[4] De La Camara, P., Fernandez Martinez, J., de Buen, E., and Juarez, E., *Medicina Paises Cálidos*, 1932, **5**, 218; from *Trop. Dis. Bull.*, 1933, **30**, 10.

[5] Francis, E., *Public Health Rep.*, 1938, **53**, 2220.

[6] Buschke, A., and Kroó, H., *J. A. M. A.*, 1923, **80**, 515.

* This strain of spirochetes was supplied through the kindness of Dr. C. M. Wheeler, Hooper Foundation for Medical Research, San Francisco, California.

[7] Beck, M. D., *J. Infect. Dis.*, 1937, **60**, 64.

greater than in hamsters infected with hamster blood and the infection remained usually from 22 to 26 days. Fatalities in the mouse blood infected group appeared less frequently; one-fifth of these hamsters died in from 12 to 37 days. In animals infected with hamster blood spirochetes appeared later, generally from 48 to 72 hours after inoculation and 3 relapses commonly occurred. More than half of the hamsters infected with blood from other hamsters died in from 9 to 30 days, usually after 2 weeks. Brains removed from untreated animals whose blood had been cleared of spirochetes for from 8 to 10 days revealed spirochetes when sections were stained according to the method of Steiner[8] (Fig. 1).

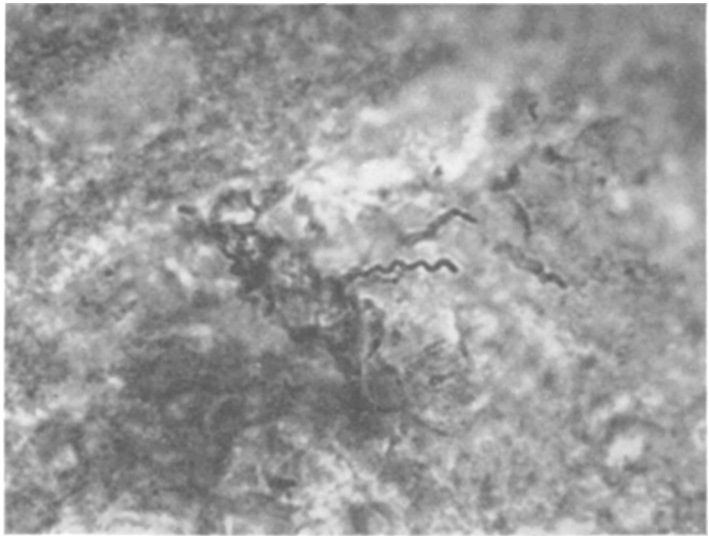

FIG. 1.
Photomicrograph of a section of the brain of an untreated Chinese hamster showing residual infection. Spirochetes are seen in the cortical gray matter. Steiner's stain. × 1940.

Since arsphenamine was first reported by Ehrlich and Hata to be effective against European strains of relapsing fever spirochetes in mice many conflicting reports have appeared.[9] Residual brain infection and so-called "arsenic-fast" strains presumably have accounted for unsuccessful therapeutic trials. In view of these conflicting reports a study of the chemotherapy of experimental relapsing fever in Chinese hamsters was attempted. Two drugs were

[8] Quoted by Spielmeyer, von W., *Technik der Mikroskopischen Untersuchung des Nervensystems*, p. 160, Julius Springer, Berlin, 1930.

[9] Quoted by Fischl, V. und Schlossberger, H., *Handbuch der Chemotherapie*, p. 488, Fischers Mediz. Buch., Leipzig, 1934.

considered: neoarsphenamine† and a less toxic arsenical, the trisodium salt of 4, 4′ dihydroxy arsenobenzene-N, N′-dimethylene sulfonic acid (Trisodarsen).‡ The LD_{50}, the dose which kills 50% of the animals, of these agents was determined, according to the method of Gaddum, *et al.*,[10] so that a therapeutic dose level might be established. Acute toxicity tests were made on 82 inbred white mice and 54 stock hamsters and chronic toxicity tests were made on 30 mice.

The acute subcutaneous LD_{50} for neoarsphenamine was found to be 235 mg/kg for mice and 190 mg/kg for hamsters. LD_{50} for trisodarsen in hamsters was 685 mg/kg. In chronic toxicity tests, 2 deaths occurred in mice after administration of an amount equivalent to LD_{50} had been given in divided doses over 10 days; no deaths occurred after equivalent amounts of trisodarsen. For treatment, 1/5 of the LD_{50} of each drug was given subcutaneously on alternate days beginning within 72 hours after the animals were inoculated. This dose was given 5 times. Examinations of the tail blood were made daily by means of thin smears prepared with Wright's stain.

A group of 30 hamsters was infected with mouse blood and another group of 30 with blood from other hamsters. Half of the hamsters of each group were treated with neoarsphenamine and the other half were given trisodarsen. In mouse blood infected hamsters given neoarsphenamine, 7 of 15 were still positive 46 hours after the second injection. Four-fifths of the LD_{50} of this drug had to be given before all animals had negative blood smears. Four hamsters developed residual infections, as shown by animal inoculations, and 2 of 15 in this group died.

Animals infected with hamster blood were rid of their spirochetes within 46 hours after the second treatment had been given. After the conclusion of therapy, however, 5 neoarsphenamine treated hamsters of this group showed residual infection by animal inoculations. Three of the 15 hamsters died within 19 days.

Hamsters infected with mouse blood responded promptly to trisodarsen; all showed no spirochetes in the peripheral blood 46 hours after the second treatment. There were 3 residual infections, as shown by animal inoculations, in this group. Two of the 15 animals died. Hamster blood infected animals responded similarly to triso-

† Purchased locally; prepared by the Abbott Laboratories, North Chicago, Illinois, U.S.A.; Lot No. 905L004.

‡ Supplied through the courtesy of the Abbott Laboratories, North Chicago, Illinois, U.S.A.; Lot No. 811L073.

[10] Gaddum, *et al.*, Med. Res. Counc., Spec. Rep. Ser. No. 128, 1929, and No. 183, 1933.

darsen in that all became negative for spirochetes after the second injection and remained free during the period of observation. Two of 15 died within 19 days. This was the only group that showed no residual infections as shown by animal inoculations.

Three procedures were utilized for confirming the results of treatment: sub-inoculation into test hamsters, examination of brain tissue by Steiner's method[§] and culture on developing eggs after the method of Oag[11] and Bohls, *et al.*[12, ¶] The results of these follow-up studies showed that 9 of the 30 hamsters treated with neoarsphenamine retained viable spirochetes in the brain as revealed by animal inoculations, while only 3 residual infections were found by this test in 30 animals after treatment with trisodarsen. Eight of 15 untreated hamsters showed residual infection by animal inoculations.

Summary. An infection with a California strain of relapsing fever spirochetes was established in Chinese hamsters which responded more satisfactorily to trisodarsen than to neoarsphenamine.

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Low Temperature and Radiosensitivity of Skin of New-Born Rats. II. Resistance at Different Dosages.*

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In a previous paper[1] it has been reported that lowering the temperature of new-born rats during irradiation (1,300 r) decreased

§ We wish to express our thanks to Dr. Y. K. Hsu, Division of Neurology and Psychiatry, for the staining and examination of brain sections and for the photomicrograph.

11 Oag, R. K., *J. Path. and Bact.*, 1939, **49**, 339.

12 Bohls, S. W., Irons, J. V., and De Shazo, T., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 375.

¶ We would like to express our gratitude to Dr. K. C. Chen, Department of Bacteriology and Immunology, for making inoculations and for the examination of cultures.

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1 Evans, T. C., *J. Roentgenol. and Rad. Ther.*, in press.