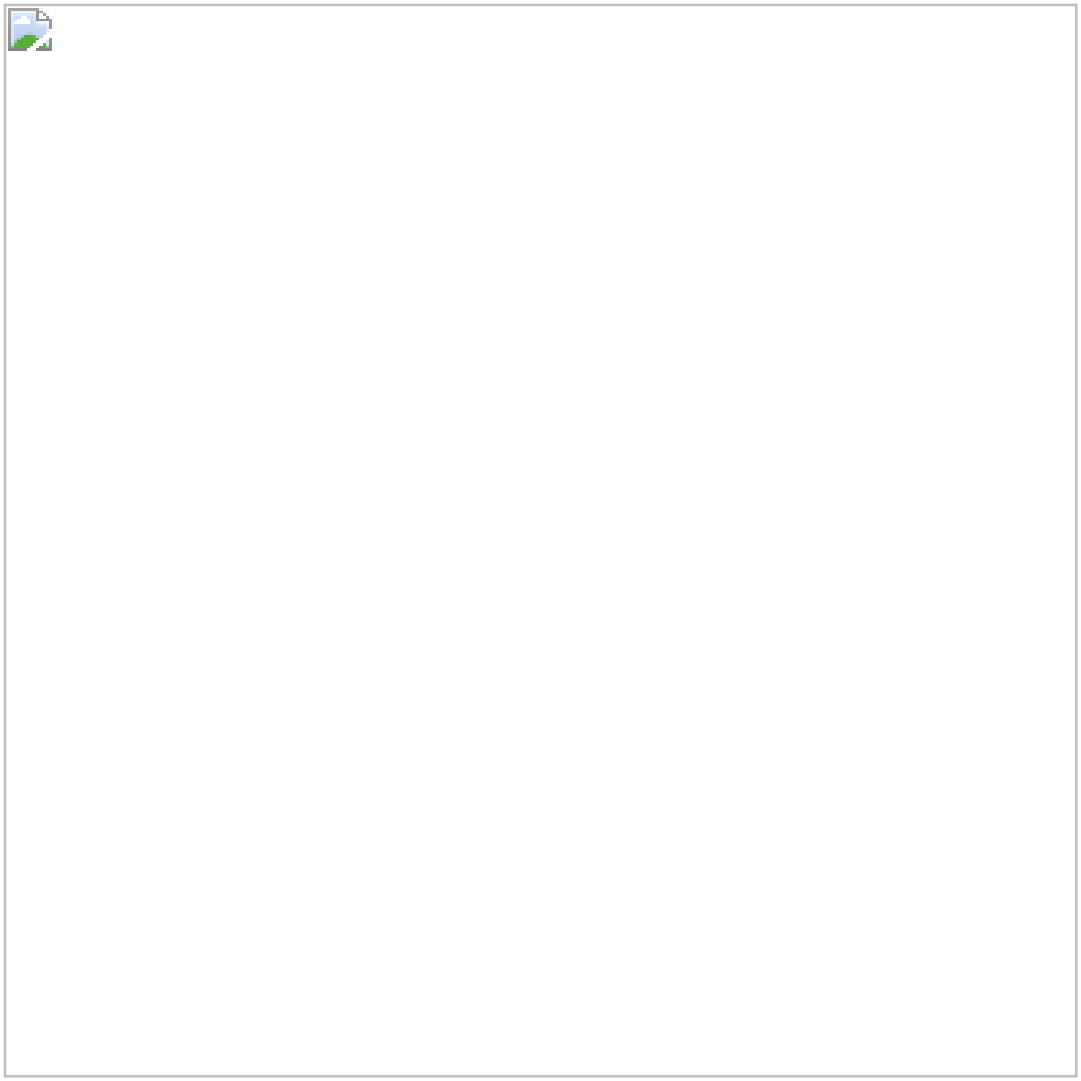

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Author: STALHEIM, O. H. V.

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A BRIEF REVIEW OF PROGRESS IN CHEMOTHERAPY USING LEPTOSPIROSIS AS A MODEL

O. H. V. STALHEIM

National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010

In searching for someone to chair this section on progress in chemotherapy of wildlife and domestic animals, our chairman selected a leptospirologist. It was a logical choice, I believe, for two reasons. First, leptospirosis occurs in a great variety of rodents and both wild and domestic animals, and therefore constitutes a rather constant danger to those of us who work with animals.[1] And secondly, progress in leptospirosis chemotherapy or the lack of it, can be used to illustrate some goals, some techniques, as well as some of the problems that still exist.

Paul Ehrlich introduced the term chemotherapy for the direct chemical attack on invading microorganisms. He called it a chemical knife and it was to be as carefully designed and as precisely applied as the surgeon's knife.[9] Leptospires cause losses in agriculture due to acute infection and death (acute leptospirosis), and abortions of fetuses (fetal leptospirosis); they also cause persistent, renal infections (renal leptospirosis). At the present time, the treatment of these three forms of the disease — acute, fetal, and renal — is accomplished in different ways. Most of the acute infections in animals and man are mild and recovery occurs spontaneously without treatment. However, chemotherapy of severe cases (i.e. classical Weil's disease) is of little or no value except perhaps in the early stage of infection.[1] During the first 4 days after exposure, both antibiotics (oleandomycin,[21] cephalothin[22]) and organic selenium compounds[7] are effective according to the results of studies in laboratory animals.

Leptospires also have the ability to invade the uterus of pregnant cows and sows and to kill the fetus. If the fetuses are uniformly susceptible, "storms" of abortions may occur with losses up to 100%. Little can be done ot curb such severe losses, but in less precipitous outbreaks, the administration of tetracycline in the feed for 10 to 14 days apparently lessened the losses.[19]

Following an acute infection, leptospires may localize in the kidneys for a few months or sometimes for the life of the animals in the case of rats. If a cheap, safe, and effective drug were available in oral form, it could be utilized, not only in herds of infected cattle and swine, but also for use in valuable foundation animals (bull studs), companion pets (dogs, mice, hamsters, etc.), and wild animals maintained in close confinement; outbreaks of leptospirosis have been recorded in zoos among monkeys,[10,13] baboons,[6] chimpanzees, and foxes.[1] If there were a cheap and simple regimen for the termination of renal leptospirosis in cattle and swine, it would be possible not only to reduce the dissemination of leptospires among animals but also the pollution of rivers and streams, and thereby reduce the hazards to persons who work with animals or who frequent recreational areas.

Because some of the methods I have adapted or developed for studies on the chemotherapy of renal leptospirosis may be applicable to studies in wildlife, I will describe them briefly and also mention some of the pitfalls.[15,16,17,18] By the use of suitable strains of *Leptospira pomona,* renal leptospirosis can be consistently initiated in laboratory animals and in cattle and swine. Two to three weeks later, the therapeutic agent is administered in the customary dosage to part of the infected animals. If the dosage has not been established, it is arbitrarily fixed. The drug is

also administered to noninfected animals as a control of drug toxicity. Two or three weeks later, when the drug has been completely eliminated, and when any persistent leptospires have multiplied to detectable concentrations, the animals are killed and the kidneys are examined for leptospires. If leptospires are not isolated from infected animals after treatment with a drug but are present in untreated infected animals, we can conclude, with certain reservations, that the drug cured the infection.

The first reservation regarding the validity of negative cultural results concerns the adequacy of current procedures for the isolation of leptospires. Although most leptospires are not difficult to grow in artificial medium, some of them are very susceptible to the leptospiricidal effect of normal serum[11] — a component of most mediums.[20] Fastidious strains can sometimes be isolated in medium supplemented with only the albumin component of serum;[8] therefore, at least two mediums, one of which contains albumin instead of whole serum,[9,5,12] should be used in isolation attempts. Furthermore, differences exist in the minimal inoculums (i.e. the minimal number of leptospires required to initiate growth in medium). They will vary for different mediums and for different batches of the same medium (Fig. 1), and they are larger for leptospires in host tissues. For example, homogenized, porcine, renal tissue lysed *L. pomona* unless the tissue suspension was extracted with ether or diluted a thousand times.[14] The rate of disappearance of *L. pomona* (i.e. lysis) when added to homogenized renal tissue is shown (Fig. 2). Because of the lytic effect of tissue suspensions, attempts to isolate leptospires by microbial cultural techniques may fail unless each gram of tissue contains 1,000 or more leptospires. During critical experiments, therefore, some investigators also test tissue for leptospires by injecting it into hamsters; two to three weeks later, they examine the hamsters for renal leptospirosis.

The second reservation regarding the validity of negative cultural results concerns the possibility that the leptospires might be modified into a resistant form such as L-phase, or that they might persist in a secluded site where they would be protected from the effects of the therapeutic agent. Then after therapy, they might emerge to repopulate the renal tubules. However at the present time, there is no evidence of either "compartmentalization" within the brain, the eye, or other organ, or of intracellularity in spirochetal or nonspirochetal form, and neither antibiotic-resistant leptospires nor L-forms have been reported.[18]

My results can be summarized briefly as follows: Although several antibiotic agents and dyes inhibited the growth of leptospires *in vitro,* only dihydrostreptomycin and possibly the tetracyclines were effective against leptospires localized in the renal tubules of hamsters. Only these, therefore, merited further trials in swine. Infected swine were treated with different amounts of dihydrostreptomycin; 10 to 14 days later, they were killed and examined for evidence of renal leptospirosis. It was found that single doses (25 mg. per kg. of body weight) were effective. Chlortetracycline was also effective when fed to swine for 10 days but only at dosages which are twice the limit permitted by the FDA. Therefore, studies on the oral medication of renal leptospirosis were discontinued.

Studies were then done on the efficacy of a single chemotherapeutic agent, dihydrostreptomycin, for the elimination of the carrier condition in cattle. All of 24 infected cattle were cured by either 3 injections, 2 injections, or a single treatment with dihydrostreptomycin, 25 mg. per kg. of body weight. Smaller doses were not tested. Leptospires were recovered from the kidneys of all but one of 11 nontreated, control cattle.

Unfortunately dihydrostreptomycin is poorly absorbed from the digestive tract and must be given by parenteral injection; therefore, the search continues for an effective chemotherapeutic agent that can be administered in the feed or drinking water to herds of cattle or swine.

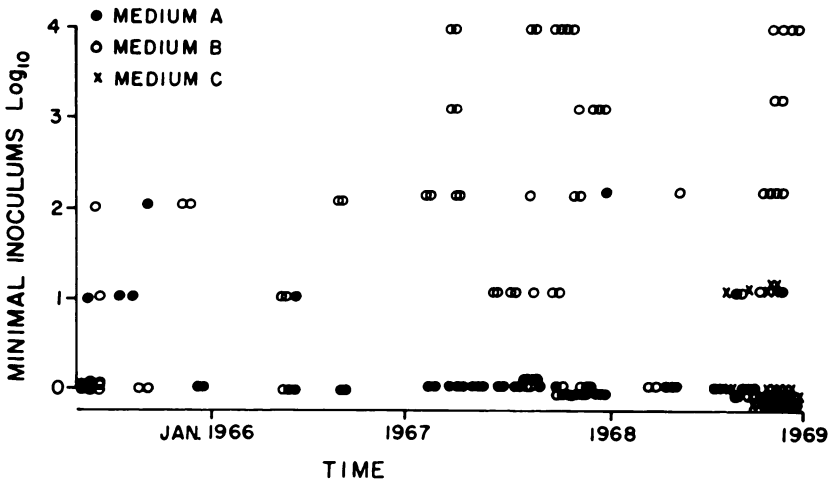

FIGURE 1. *A comparison of minimal numbers of leptospires required to initiate growth in 1 milliliter of three mediums. Numbers of viable* Leptospira pomona *strains DM, DM-2, Wickard, MLS, and GLD in infected kidneys or cultures were estimated by viable counts*[12] *in different lots of different mediums and compared as minimal inoculums per milliliter.*

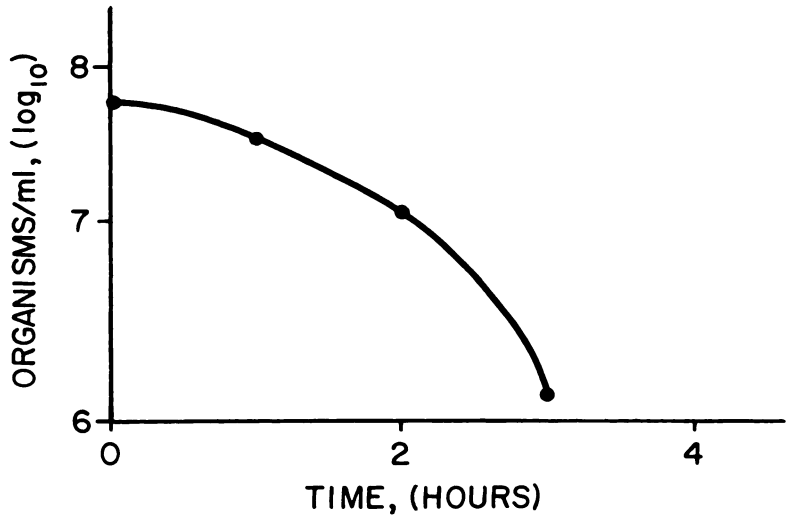

FIGURE 2. *Lysis of* L. pomona *by homogenized renal tissue. Microscopic counts were made after mixing* L. pomona *with a 20% suspension of porcine tissue.*[13]

Since the problems of chemotherapy for the control of disease appear formidable indeed, involving as they do chemistry, microbiology, therapeutics, and clinical sciences, perhaps it would be helpful to notice how Paul Ehrlich approached the problem. In his book, *Experimental Chemotherapy of Spirilloses,*[4] he remarks that you cannot catch fish with a net in a wide river unless the net is stretched straight across. He describes how he stretched his net of chemists, biologists, and clinicians across the stream and caught the first "big fish," 606 or arsphenamine. There has been progress in chemotherapy for the control of wildlife diseases (e.g. psittacosis),[2] but perhaps progress would be facilitated by the use of a modern version of Ehrlich's multidiscipline net.

Literature Cited

1. ALSTON, J. M., and J. C. BROOM. 1958. Leptospirosis in Man and Animals. E. and S. Livingstone Ltd., Edinburgh and London.
2. ARNSTEIN, P., B. EDDIE, and K. F. MEYER. 1968. Control of Psittacosis by Group Chemotherapy of Infected Parrots. Am. J. Vet. Res. 29: 2213-2227.
3. EHRLICH, P. 1909. Uber den Jetzigen Stand der Chemotherapie. Berlin d. Deutsch. Chem Gesellsch. 42: 17-47.
4. EHRLICH, P., and S. HATA. 1911. The Experimental Chemotherapy of Spirilloses. Trans. A. Newbold. Rebman, London.
5. ELLINGHAUSEN, H. C., JR., and W. G. McCULLOUGH. 1965. Nutrition of *Leptospira pomona* and Growth of 13 Other Serotypes: Fractionation of Oleic Albumin Complex and a Medium of Bovine Albumin and Polycorbate 80. Am. J. Vet. Res. 26: 45-51.
6. FEAR, F. A., M. E. PINKERTON, J. A. CLINE, F. KRIEWALDT, and S. S. KALTER. 1968. A Leptospirosis Outbreak in a Baboon (Papio Sp.) Colony. Laboratory Animal Care 18: 22-28.
7. GOBLE, F. C., E. A. KONOPKA, and H. C. ZOGANAS. 1968. Chemotherapeutic Activity of Certain Organic Selenium Compounds in Experimental Leptospirosis. Antimicrobial Agents and Chemotherapy. 1968, p. 531-533.
8. HANSON, L. E., H. C. ELLINGHAUSEN, and RACHEL MARLOWE. 1964. Isolation of *Leptospira grippotyphosa* from a Cow Following an Abortion. Proc. Soc. Exptl. Biol. Med. 117: 495-497.
9. IMAMURA, S., Y ASHIZAWA, and K. YONEYAMA. 1962. Cultviation of Leptospirae. I. Japanese Jour. Bacteriol., 17: 749. (In Japanese.)
10. JOHNSON, PETER, JR. 1969. Leptospirosis in a Monkey Colony. Zoo Veterinarian. (In Press.)
11. JOHNSON, R. C., and L. H. MUSCHEL. 1966. Antileptospiral activity of serum. I. Normal and immune serum. J. Bacteriol. 91: 1403-1409.
12. JOHNSON, R. C. and V. G. HARRIS. 1967. Differentiation of Pathogenic and Saprophytic Leptospires. I. Growth at Low Temperatures. J. Bacteriol. 94: 27-31.
13. SHIVE, R. J., S. S. GREEN, L. B. EVANS, and V. M. GARNER. Leptospirosis in Barbary Apes. Jour. AVMA. (In Press).
14. STALHEIM, O. H. V. 1965. Leptospiral Lysis by Lipids of Renal Tissue and Milk. J. Bacteriol. 89: 545.
15. STALHEIM, O. H. V. 1966. Effects of Antimicrobial Agents on Leptospiral Growth, Respiration, Motility, and Viability. Am. J. Vet. Res. 27: 797-802.
16. STALHEIM, O. H. V. 1966. Chemotherapy of Renal Leptospirosis in Hamsters. Am. J. Vet. Res. 27: 803-807.

17. STALHEIM, O. H. V. 1967. Chemotherapy of Renal Leptospirosis in Swine. Am. J. Vet. Res. 28: 161-166.

18. STALHEIM, O. H. V. 1969. Chemotherapy of Renal Leptospirosis in Cattle. Am. J. Vet. Res. (In Press).

19. STOENNER, H. G. 1968. Bovine Leptospiral Abortion. *In* Lloyd C. Faulkner (Editor), Abortion Diseases of Livestock. Charles C. Thomas, Springfield, Ill.

20. STUART, R. D. 1946. The Preparation and Use of a Simple Culture Medium for Leptospirae. J. Pathol. Bacteriol. 58: 343-349.

21. WOOD, G. E., and A. D. ALEXANDER. 1960. The Effect of Selected Antibacterial and Antiprotozoal Agents in Treatment of Leptospirosis in Experimentally Infected Hamsters. Antibiotics and Chemotherapy X: 341-348.

22. YOSHIMORI, R. N., H. S. GOLDBERG, and D. C. BLENDON. 1966. Cephalothin in the Treatment of Experimental Leptospirosis in Hamsters. Antimicrobial Agents and Chemotherapy — 1965, p. 450-452.