of interest that a small but definite fraction of the radioactivity was recovered as sulfate and as organic sulfur from feces and intestinal contents of rats given the compound intraperitoneally. The activity of the feces in these experiments could have been due to contamination with urine despite precautions employed to prevent such contamination; this could not be true in the case of the intestinal contents, since these had never been in contact with urine.

It is apparent that, while a small portion of the sulfur of isocysteine hydrochloride given to rats either by stomach tube or by intraperitoneal injection may be oxidized to sulfate and excreted as such, a much larger fraction is still bound in organic combination when it is excreted. The rat, therefore, reacts similarly to the rabbit in that it does not readily oxidize the sulfur of isocysteine to sulfate.

Quantitative differences between the two species may, however, exist. The rats employed in this investigation excreted in the urine, within 24 hours, 54 to 67% of the S^{35} administered as isocysteine by stomach tube. The rabbits employed in the earlier study[1] excreted, within 24 hours, "extra" sulfur equivalent to only 29 to 37% of the dose given by stomach tube. On the other hand, the rabbits excreted "extra" sulfur equivalent to 76 to 98% of the dose when isocysteine was administered subcutaneously; rats excreted only about 52% of the S^{35} of isocysteine which had been injected intraperitoneally. The metabolic fate of the S^{35} not found in the excreta or intestinal contents of these rats cannot be ascertained from the present data, but presumably it was still contained in the tissues.

Summary. 1. The oxidation of the sulfur of isocysteine by the rat was studied with the aid of isocysteine containing S^{35}.

2. It was found that only a small amount of S^{35} was excreted as sulfate in the urine within 24 hours after administration of the compound by stomach tube or by intraperitoneal injection. A much larger portion was excreted as organic sulfur. A very small fraction of the S^{35} was recovered in the feces and intestinal contents of the rats.

3. The results of the present study are compared with those of an earlier investigation in which rabbits served as subjects.

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16957

Streptomycin in the Treatment of Leptospira Carriers. Experiments with Hamsters and Dogs.*

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The carrier problem dominates the control of canine leptospirosis. Dogs remain urinary shedders for from 2 to 6 months after recovery. With the introduction of chemotherapy, the risk of creating carriers and thus spreading leptospirae is greatly increased.

Penicillin and streptomycin have been found to inhibit growth of leptospirae *in vitro* and to be effective against experimental leptospirosis in guinea pigs, mice and hamsters. Shih Lu Chang[1] observed that *Leptospira icterohaemorrhagiae* disappeared from the blood of guinea pigs 3 to 5 days after 2 daily injections of aqueous solution of penicillin in total daily doses of 800 units. A

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1 Chang, S. L., *J. Clin. Invest.*, 1946, **25**, 752.

serum concentration of more than 0.2 unit per cc was maintained. However, the leptospirae persisted in the liver even when the dose was increased to 3,000 units daily and continued for 6 to 8 days after the blood tests had become negative. Wylie and Vincent[2] found that unless penicillin was given to the guinea pigs 24 hours after inoculation of *L. icterohaemorrhagiae*, the guinea pigs died. These authors stated that renal damage might have made the leptospirae inaccessible to the antibiotic.

Petersen and Schmidt[3] demonstrated leptospirae in the kidneys of a guinea pig 3 months after inoculation with *L. icterohaemorrhagiae* and penicillin treatment. In experiments reported by Brunner,[4] hamsters were proven renal carriers 26 days after inoculation with *L. canicola* and penicillin treatment. In discussing these results it was emphasized that although penicillin treatment may save the life of a hamster or a dog, it may favor the development of a carrier state.

Heilman and Herrell[5,6] successfully treated hamsters infected with *L. icterohaemorrhagiae* with streptomycin. Treatment was started 17 hours after inoculation, and no leptospirae were found in the livers and kidneys of the treated animals after 28 and 33 days. In hamsters infected with *L. canicola* and treated with streptomycin, Brunner failed to demonstrate the organisms in the kidneys by culture and darkfield examination.

It would appear from the foregoing that penicillin is only effective in leptospiremia, while streptomycin either prevents the leptospirae from reaching the renal tubules or is able to destroy them there. The important question of whether leptospirae multiplying in the renal tubules of chronic carriers could be reached and destroyed by streptomycin had not been answered by these studies. It is the purpose of this paper to record experiments in this direction.

Experimental work. Series 1. Forty hamsters, 4 or 5 weeks old, were each injected intraperitoneally with 0.25 cc of a virulent *Leptospira canicola* culture (120,000 organisms per cu mm). On the 60th hour after injection each hamster was given intramuscularly 500 mg of penicillin in oil and wax, and a second dose of 250 mg 12 hours later. Four hamsters died of leptospirosis after 12 to 15 days. Thirty-two days after inoculation, 9 of the 36 surviving hamsters were killed, and kidney material was examined in the dark field and cultured. All the 9 cultures were positive for leptospirae, and in 7 cases could the organisms readily be demonstrated by darkfield illumination in the fresh specimens of kidney.

Having established that the hamsters were carriers, 18 of the series were treated on 3 successive days with 5 mg of streptomycin each intramuscularly, given in oil and wax (0.1 cc volume), and 9 hamsters were left untreated as further controls. The 18 treated hamsters and the 9 untreated controls were killed on the 2nd day after the last streptomycin treatment, and kidney material was examined microscopically in the dark field and cultured. All the 18 cultures of kidney material of the treated animals were negative for leptospirae and remained sterile, whereas the cultures of the 9 controls were all positive, and in 8 the organisms could be demonstrated on darkfield examination.

In summary, then, of 36 hamsters inoculated with a virulent culture of *L. canicola* and treated with penicillin, 18 chosen at random and killed 32 to 37 days after inoculation were renal carriers, while the remaining 18 treated on 3 successive days with streptomycin were proved to have sterile kidneys 2 days after therapy had been instituted on the 33rd day after infection.

Series 2. Five dogs (about 4 months old) were infected intraperitoneally with a virulent culture of *Leptospira canicola* (about 50 million organisms per kilo body weight). Eighteen days after inoculation large numbers of leptospirae were found in the urine of all the

[2] Wylie, J. A. H., and Vincent, E., *J. Path. and Bact.*, 1947, **59**, 247.

[3] Petersen, B. C., and Schmidt, R. M., *Acta path. et microbiol. Scandinav.*, 1945, **22**, 462.

[4] Brunner, K. T., *California Veterinarian*, 1948, **1**, 18.

[5] Heilman, F. R., *Proc. Staff Meet., Mayo Clin.*, 1945, **20**, 169.

[6] Heilman, F. R., and Herrell, W. E., *Proc. Staff Meet., Mayo Clin.*, 1944, **19**, 89.

dogs. (One dog died during the 5th week; the findings at autopsy were typical of leptospirosis.) The urine of one of the carriers was again examined 4 weeks after inoculation and found to be heavily contaminated with leptospirae. The dog (8.5 kg) was then treated with streptomycin, 700 mg on the 1st day and 500 mg on the 5 following days, given intramuscularly in oil and wax. One day after the last treatment, results of urinalysis for leptospira were negative; the same day leptospirae were found in the urine of the 3 remaining carriers. The pH values of the urine were found to fluctuate between 6 and 8; living leptospirae were not found at pH values below 7 (the dead organisms could still easily be demonstrated in the dark field). Care was taken to obtain neutral or alkaline samples from all the dogs.

The 3 untreated carrier dogs were then treated with streptomycin 3 days after urine had again been examined and organisms found (5 weeks after inoculation of the culture). The dose was 40 mg per kilo body weight for 4 days, given intramuscularly in oil and wax. On the 5th day after the beginning of treatment, organisms were not found in the urine (4 dogs). Darkfield examinations were made and urine was injected intraperitoneally into young hamsters.

Urine from the 4 treated dogs was again examined 11 days later and organisms were not found. The 4 dogs were then sacrificed and the kidneys examined, kidney material cultured and injected intraperitoneally into young hamsters. Results of these examinations were negative.

Series 3. Four dogs (4 months old) were given intraperitoneal injections, 2 with a virulent culture of *L. canicola* (about 120 million organisms per kilo body weight) and 2 with virulent cultures of *L. icterohaemorrhagiae* (about 30 million organisms per kilo body weight). One dog infected with *L. canicola* died on the 6th day of leptospirosis. Urine of the dogs was examined microscopically in the dark field 14 days after inoculation and found to contain large numbers of leptospirae. A second urine examination was made 9 days later, and results were again strongly positive. On the same day, streptomycin treatment of 2 dogs was started; 1 dog infected with *L. icterohaemorrhagiae* was left untreated. The daily dose used was 40 mg per kilo body weight given intramuscularly in oil and wax on 3 successive days. Two days after the last treatment urine from all 3 dogs was examined, and organisms were found only in the urine of the control dog. The 2 treated dogs were sacrificed, kidney material cultured and examined in the dark field with negative results.

Conclusions. The data here presented prove that chronic renal infections with leptospira in hamsters and dogs may be successfully cured with streptomycin. Although penicillin, as well as streptomycin, effectively influences the leptospiremia in experimental infections in guinea pigs, mice and hamsters, only streptomycin is capable of destroying the organisms found in the convoluted tubules.

Canine leptospirosis is usually treated after the parasite has already disappeared from the blood. Obviously, penicillin is of little value. Streptomycin in oil and wax in a daily dose of 40 mg per kilo given intramuscularly should be used in the treatment of acute and chronic canine leptospirosis. In fact, this antibiotic may be employed in the prevention of this destructive disease in dogs. It is recommended that the urine of all dogs intended for breeding be examined for leptospira, and when infection is found, the dogs should be treated for 3 to 5 days with streptomycin a few days before the act of breeding.