lines are in progress. It is interesting to note that the findings set forth in the present paper regarding rabbits injected with large doses of cobra venom over long periods of time agree with the clinical observations of Steinbrocker, McEachern, La Motta and Brooks[10] in their study on human subjects with material supplied by the senior author of the present paper. These findings agree also with the undetailed report recently published concerning the work of French investigators, who found that no change was produced in the blood sugar level of rabbits but that a rise in blood sugar of guinea pigs was effected by injections of a cobra venom preparation, regarding which details are not given.[11]

Summary. Large quantities of cobra venom were injected in a series of rabbits for periods varying from 2 to 21 weeks. Morphological and biochemical studies on the blood revealed no striking pathological change and no specific effect on the blood picture of the animals as compared with normal controls.

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Toxicity for Dogs of a Bactericidal Substance Derived from a Soil Bacillus.

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(Introduced by Rene J. Dubos.)

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Dubos[1] has described the isolation from a sporulating soil bacillus of an agent which exerts a powerful bactericidal effect *in vitro* upon Gram positive bacteria, and which if injected intraperitoneally affords protection to white mice infected by the same route with pneumococci or hemolytic streptococci. Subsequently Dubos and Cattaneo[2] reported the results of studies with a protein-free preparation of the bactericidal agent which duplicated the earlier results obtained with the crude protein-containing extract. The present report deals with the toxicity for dogs of a more highly purified protein-free preparation of the bactericidal agent injected by the intravenous route. The

10 Steinbrocker, McEachern, La Motta and Brooks, *J. A. M. A.*, 1940, **114**, 318.
11 Foreign Letter from Paris, *J. A. M. A.*, 1940, **114**, 425.
1 Dubos, R. J., *J. Exp. Med.*, 1939, **70**, 1; Dubos, R. J., *Ibid.*, 1939, **70**, 11.
2 Dubos, R. J., and Cattaneo, C., *J. Exp. Med.*, 1939, **70**, 249.

preparation used was active both *in vitro* and *in vivo* (mice) against Gram positive bacteria.

Short-haired dogs weighing from 8.0 to 12.0 kg were chosen. Prior to the course of injections the animals were observed for from 1 to 2 weeks, and only those showing a normal temperature curve, normal urinalysis and normal blood picture were used. Blood counts and urinalyses were performed daily during the course of injections and at intervals thereafter in the animals which survived. Temperature readings were taken at least twice daily and the animals were weighed frequently throughout the period of observation. Pathological study was made of all dogs which received injections of the bactericidal substance; those animals which did not die as a result of the injections were sacrificed at intervals following treatment. The bactericidal substance was dissolved in alcohol and dilutions of the alcoholic solution in 20 cc of a 5% glucose solution in redistilled water were used for intravenous injection. The doses ranged from 0.05 to 2.0 mg per kg (mg/kg) of body weight per day and injections were continued for 10 days in the dogs which survived. (Fig. 1.)

Six of the 8 animals receiving 0.4 mg/kg or more per day died before the full course of injections was completed, the total dosage varying betwen 1.5 mg and 5 mg/kg. One dog receiving 0.5 mg/kg died on the 42nd day after the course of injections was begun, and one animal which survived the 10 daily injections of 0.4 mg/kg was sacrificed on the 33rd day. None of the 5 dogs died which received a dosage of 0.3 mg/kg or less for 10 days. These were sacrificed

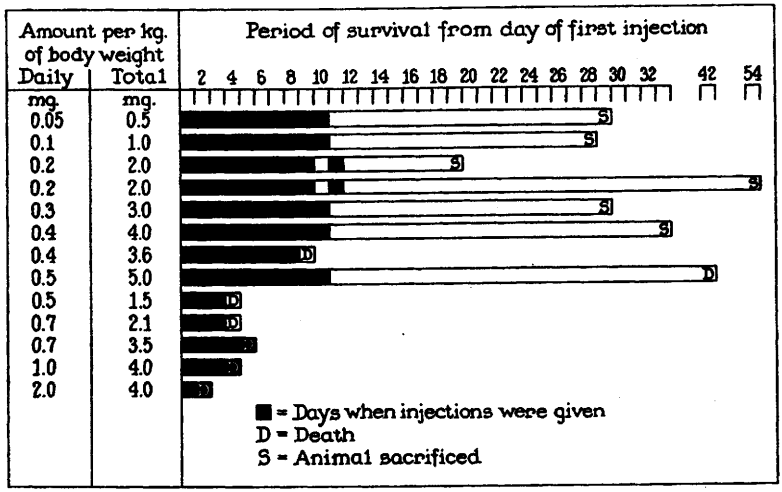

FIG. 1.

Period of survival of dogs receiving varying amounts of the bactericidal substance.

from 23 to 54 days after the course of injections was begun. All of the animals receiving 0.3 mg/kg or more per day showed well-marked toxic manifestations of either acute or chronic nature. In the animals receiving 0.2 mg/kg or less the evidence of toxicity was of minor degree.

The more prominent signs of toxicity were loss of weight associated with anorexia, fever which occurred particularly following the first few injections of the agent, progressive anemia, ascites, hematuria and the excretion of bile in the urine. In the animals which died acutely following injection of the larger doses (0.5 to 2.0 mg/kg) there was marked congestion present in the lungs and abdominal viscera with petechial hemorrhages in the heart, lungs and kidneys. The livers showed acute central necrosis associated with hemorrhage and dilatation of the sinusoids. Diffuse hemorrhage occurred in the spleen, with pronounced phagocytosis of red blood cells by the macrophages. In the kidneys hemorrhage was most marked in the glomeruli. In the animals which received daily 0.3 mg/kg or more and which did not die acutely, the changes in the organs were of a more chronic nature. The liver cells showed fatty degeneration which was most marked about the central veins. In these areas there was an increase in the reticular tissue, but cirrhotic changes were minimal. In 2 of the animals ascites was present. The only change noted in the organs of the animals receiving 0.2 mg/kg or less was a slight degree of fatty degeneration of the liver.

Summary. A study has been made of the toxicity for dogs of a protein-free preparation of the bactericidal substance described by Dubos when injected by the intravenous route. Seven of the 8 animals which received 0.4 mg/kg or more daily died as a result of the injections, and in 6 of these death occurred before the course of 10 daily injections was completed. All animals receiving 0.3 mg/kg or more showed well-marked acute or chronic changes in the liver, spleen, kidneys, heart and lungs. Animals which received daily 0.2 mg/kg or less for 10 days showed only minor evidence of toxicity.