

in 5 minutes. Three kittens were given a similar quantity of the toxin after it had been heated for 2 hours at 100°C. and none vomited. A majority of the kittens were drowsy and weak after each of the inoculations. One that received the control medium had a diarrhea after 24 hours. The 3 kittens given the staphylococcal toxin grew progressively weaker and all were dead after 4 hours. The pathological changes were similar to those in the puppies.

The fact that the kittens and puppies were sick, vomited, and had a diarrhea following the intraabdominal injection of 5 cc. of staphylococcal toxin indicates, according to Dolman's test, the presence of an enterotoxin. Three kittens given 5 cc. of heated toxin and 2 dogs given an equal volume of the same toxin and 2 other dogs given twice this amount did not vomit. Four out of a group of 5 puppies given 5 cc. of the control medium vomited within a period of 30 minutes. Of a group of 7 kittens given the control medium, 2 vomited.

Since some of the kittens and dogs in this study were sick and vomited following injection of the control medium it would appear impossible to determine whether or not they vomited from the effect of the toxin or from the effect of the medium. Furthermore it does not seem correct to conclude that staphylococcal enterotoxin is thermostable. In this study none of 4 dogs or 3 kittens vomited after receiving the heated toxin.

The volume of the material injected and the individual variation in animals of the same species may account for the observations that have been made in an attempt to separate the staphylococci into 2 groups: those which do and those which do not produce an enterotoxin.

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Cataphoretic Separation of Toxic Components of Moccasin Venom.

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Attempts at separation of the various active components of snake venoms have not been very successful. These principles are probably proteins or substances closely related to proteins.¹ They are quite

¹ Peck, S. M., and Marx, W., publication in preparation.

unstable.^{1, 2} For this reason, chemical procedures used in their separation, such as precipitation, extraction or absorption often resulted in the destruction of the less stable elements.

Separation of the hemorrhagic and hemolytic components of moccasin venom by cataphoresis was attempted because it was thought that this procedure might be less destructive. This method had been tried by others without success in an attempt to separate the neurotoxic and coagulant principles of *Bothrops* venom³ and the neurotoxin and hemolysin of cobra venom.⁴

In the present studies, a cataphoresis chamber was used as described by Todd.⁵ An electrical potential of 120 volts D.C. was applied to solutions of moccasin venom for 3 to 5 hours at different hydrogen ion concentrations. The liquids in the anode and cathode chambers were then tested for hemorrhagin and hemolysin.

The hemorrhagin was assayed by using the intradermal venom test in rabbits.² It was found that at pH values of 6 and above, the hemorrhagin migrated only to the anode, below pH 4 only to the cathode. Very little migration occurred in the neighborhood of pH 4-5. This indicated that the isoelectric point of the hemorrhagic component of moccasin venom was between pH 4 and 5.

The assay for hemolysin was carried out on agar blood plates.² This test was recently improved by using plain agar instead of agar plus beef broth as medium.¹ With this improved blood plate method it was possible to show that moccasin venom contained 2 different hemolytic components, and to demonstrate their separation. They were designated as hemolysin A and B.

Hemolysin A was identical with the hemolytic component which had been discussed in our first publication.² This caused the appearance of dark olive to black areas of lysis on blood plates. It was quite unstable and was rapidly destroyed outside of the pH range of 5-7. Hemolysin A was found to have an isoelectric point of approximately pH 5. At pH 6 and 7 it migrated only to the anode during cataphoresis.

Hemolysin B, the other hemolytic component, differed markedly in its lytic properties from hemolysin A. When the original blood plate method for demonstrating hemolysin was used, its hemolytic action was hardly visible. For this reason it was not discussed in our first report.² By the use of the improved blood plate method,

² Peck, S. M., and Marx, W., *J. Pharm. Exp. Therap.*, 1937, **60**, 358.

³ v. Klobusitzky, D., *Arch. Exp. Path.*, 1935, **179**, 204.

⁴ Ghosh, B. N., and De, S. S., *Ind. J. Med. Res.*, 1937, **24**, 1175.

⁵ Todd, C., *Brit. J. Exp. Path.*, 1927, **8**, 369.

however, its action could readily be demonstrated, but only in the absence of hemolysin A. The action of hemolysin B became manifest as a colorless transparent spot of lysis on the blood plate. During cataphoresis, hemolysin B always migrated to the cathode, independently of the pH, in the studied range from 3 to 8.

Since hemolysin B could not be demonstrated by the blood plate method in the presence of hemolysin A, a different technique had to be employed for its determination. This consisted in the titration of the minimal hemolysin concentration which produced complete hemolysis of a suspension of rabbit blood cells under defined conditions, lecithin being used as activator. Lecithin only activated hemolysin B. The method will be described later.¹ By this means it could be confirmed that, during cataphoresis, hemolysin B migrated only to the cathode in the studied pH range. The anode liquid was practically always negative for hemolysin B when tested in this way.

It was thus possible to demonstrate that moccasin venom contained 2 different hemolytic components which could be separated by cataphoresis: At pH 6 and 7, hemolysin A migrated only to the anode, hemolysin B only to the cathode. It could also be shown that in the same pH range, the hemorrhagin and hemolysin B could be separated by cataphoresis. The hemorrhagin migrated only to the anode, the hemolysin B only to the cathode.

Our findings seem to indicate that the molecules responsible for the action of hemolysin B are different from those which give rise to the other toxic effects of moccasin venom so far studied. Hemolysin B has a much more basic character.

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Protection of Rabbits Against Naturally Acquired Infectious Myxomatosis by Previous Infection with Fibroma Virus.

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It is generally agreed that the viruses of rabbit fibroma and rabbit myxoma are closely related immunologically and that infection with the benign fibroma virus usually protects rabbits against fatal infec-