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DEPARTMENT OF THE ARMY
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1. The physiological and pharmacological effects of subcutaneous injection of the poison of Ancistrodon halys caraganus in white mice are similar to those produced by the poison of Ancistrodon blomhoffii ussulensis. These effects are described.

2. The lethal dosage for small mice amounted to 0.1 to 0.2 mg (dry weight) of the snake venom. For rats the lethal dosage was 1.5 mg, for guinea pigs 1.5 to 2.0 mg. A tissue constricting effect was observed in an isolated ear in a dilution of $1 : 10^2 - 1 : 2 \times 10^3$. Similar effects are attained by injection in the abdominal aorta. In a dilution of $1 : 1000$ the venom has a definite hemophilic effect.

3. The tonus of the isolated frog muscle is slightly reduced by bee poison (10-100 ppm) and by toad venom (20-1000 ppm). Scorpion poison (100-2000 ppm) produces a more rapid reduction of the tonus. The venom of the pit viper ("Pallas Pit Viper," 50-5000 ppm) and the Vipera lebetina (100-10000 ppm) do not have such an effect. Repeated administrations weaken the effects of bee or toad poison. These poisons reduce the acetylcholin effect or render it impossible.

4. The poisons of bees, toads and the Pallas pit viper (100-1000 ppm) show no cholinesterase effect upon acetylcholin and no reciprocal effect with cholinesterase. Therefore its neurotoxic effect, particularly its influence upon the transmission of stimuli to the peripheral and central synapses, cannot be related to the effect of cholinesterase. One apparent exception is cobra venom. The experiments were conducted in vitro, therefore there is no connection with the possibility that acetylcholin can be deactivated by ATP metabolism.
5. Individual dosages of the poisons of wasps, hornets, bumble bees, scorpions, toads (1:250), carpet viper (1:750), cobra, Pallas pit viper (1:250), adder and viper lebentina (1:500) produced an increase of hemoglobin and erythrocytes in mice. After three to five hours a decrease took place, reaching a minimum after four to six days, followed by a gradual return to normal value. The behavior of additional values characteristic of the blood picture are also reported. Sphenektomy changes the effects of the poisons of toads, scorpions, adder, cobra and hornets in no way. The liver and spleen of mice, however, contained hemoglobin decomposition products after a lethal dosage of pit viper venom. These poisons produce little or no hemolysis; bee poison has a hemolytic effect even in a dilution of 1:1000.

6. The poisons of the following snakes were investigated as regards their effect upon catalysis:

- cobra (Naja naja) for the family colubridae
- vipera raddei and vipera lebetina for the family viperidae
- ancistrodon blomhoffi for the family crotalidae.

Dried venom was used and added to blood catalysis preparations in a concentration of 0.1 - 0.0016 mg/ml.

Cobra venom activated the catalysis within a wide range of concentrations. The effect increased with increased temperature. The poison of the viperidae and crotalidae had a pronounced arresting affect upon the blood catalysis. Differing from viperidae poison, that of the crotalidae increased the effect of blood catalysis in concentrations of 0.013 - 0.0033 mg/ml. This effect decreased with increasing temperature. Addition of snake venom varied the temperature coefficients of H₂O₂ decomposition through blood catalysis.

7. The poison of C. perfringens (Type A) had no specific effect in vitro on catalase and peroxidase from rabbit blood. In the case of anemia which was produced in rabbits by administration of lethal and sublethal doses of the poison, the catalase index was noticeably increased. The same effect is produced by injection of saponin and water. If a sublethal dosage of the poison of ancistrodon blomhoffi is given, a light temporary anemia is induced; the catalase index tends to fall in this case. Cobra venom had practically no influence upon the catalase index and blood corpuscles. The change in peroxidase activity corresponds to the change in hemoglobin content.

8. Forty eight patients who had been bitten by poisonous animals were examined. The overall protein content of the
blood remained normal; no relationship could be recognized between protein concentration and clinical findings. Following the bite of the vipera lebetina and during the development of the infection the protein content also remained normal initially but dropped between the third and eighth day. In the initial stage a drop in the α and globulins was found; later the same for the β globulins. In severe cases the protein concentration returned to normal values after seven to ten days. Only the globulin concentration increased as the result of scorpion bites.

9. A marked detoxifying effect was noted when propyl gallat was injected in mice together with the administration of snake poison. If propyl gallat was injected immediately after the poison, an unreliable protective effect could be observed. But if animals which had received propyl gallat together with snake poison two months previously were again given poison they survived the experiment.

10. Poisons of vipera lebetina, echis carinatus, Haja oxiana and a South American rattle snake were investigated. Phosphodiesterase and 5'-nucleotidase were found in all poisons. The activity of the phosphodiesterase was always 200-300 times greater than that of the 5'-nucleotidase. The greatest activity of phosphodiesterase was found in the poison of the vipera lebetina, the lowest in that of echis carinatus. The activity of 5'-nucleotidase was approximately equal in all poisons.

The content in phosphodiesterase precipitated with acetone was 8.0 to 11 percent in the poisons of V. lebetina and E. carinatus, but only 2.5 percent in the poison of N. oxiana. The separation of both enzymes by acetone precipitation gave satisfactory results only in the case of the poison of E. carinatus.

11. After administration of the poison to rabbits, the electroencephalogram and respiration changed rapidly.

12. The 5'-nucleotidase can be completely separated with a 4.8 molar solution of ZnCl₂. In the residue one can find 91-93% of the phosphodiesterase originally present which is released through chromatography or dialysis of Zn⁺⁺. Both enzymes can be dissimilarly deactivated by heating to 60° in the presence of (NH₄)₂SO₄.

13. Prior to injection of the snake venom, mice were protected by subcutaneous injection of propyl gallat. All animals survived the poison of vipera lebetina if propyl gallat was given within 1-6 minutes thereafter. An injection after
7-10 minutes led to a survival of about 20 percent. If the interval exceeded fifteen minutes, all of the animals died.

The poison of V. Renardi was more toxic. In this case, certain protection was achieved only if propyl gallat was injected within two minutes. Even more toxic were the poisons of L. carinatus and anistodron blomhoffii which produced a mortality of 20 percent after only 1-2 minutes. This method of determining the toxicity of snake venom is more reliable than that method which determines the amount of poison necessary to produce certain effects. This method yields an irreversible toxicity series for the poisons named.

(Attachment 2)

1. The poison of the vipera berus has a more marked effect of inhibiting coagulation of human blood in vitro than the poison of the vipera ammodytes. These effects are similar to those of thrombokinase from the brain of rabbits. Both poisons have considerable stability (28 days at 150°C).

2. Reptilase (a highly purified mixture of poisons from bothrops jararaca and ladesis atrox) (I), the poison of the vipera russelli (II) and a mixture of poisons from V. Russelli, V. ammodytes and anistodron piscivorus (III) were studied as to their ability to inhibit blood coagulation. I had an effect of inhibiting coagulation similar to thrombin (also like the poisons of B. atrox, B. jararaca, B. nummifera, crotalus adamanteus) and should be effective in practically all cases of coagulation disturbances provided that no shortage of fibrinogen exists. II and III showed an effect similar to that of thrombokinase, as do the poisons of V. berus and V. ammodytes.

3. A good chromatographic separation of the protein components of the poison of the V. ammodytes on paper strips was achieved through elutriation with salt solutions or buffer solutions of varying concentrations. The advantages of this method over usual development with solutions of constant composition are pointed out.

4. After centrifuging and filtration through G4 frits, whatman No. 1 and Seitz filters, the ultraviolet spectra of a 0.1 percent solution of the poison show different extinction maxima in each case. The solution was only slightly toxic after filtration with a Seitz filter No. 1011 and it had no hemolytic activity. The water unsoluble portion of the poison showed only one fifth of the hemolytic, but the same toxic effect as the original poison. Seven protein fractions were isolated by paper chromatography and examined by an electro-
phoretic micromethod. Two of the proteins show isoelectric points above pH 8.6. The others are acid.

5. The proteins of the poison of the V. ammodytes were separated at the isoelectric points (pH 5.2-5.5) by precipitation with ethyl alcohol. Following hydrolysis, the amino acids were determined by paper chromatography. Six to seven components could be differentiated through electrophoresis. The toxic principle was increased four-fold in a still impure fraction.

6. Seven protein components of the poison were separated electrophoretically. The phosphatidase-A activity can be traced to one of the separated components, the position of which in the electrophorogram is given. None of the components demonstrated hemolytic activity.

7. Seven protein components in the poison could be differentiated electrophoretically, thus confirming earlier findings. A chromatographic analysis of the protein hydrolysate revealed the following amino acids: cystin, glycine, threonin, alanin, lysin, arginin, tyrosin, valin, prolin, methionin, leucin, phenylalanin, tryptophan. By adding ethyl alcohol to an aqueous solution of the poison at 10 up to 57 percent alcohol concentration, an alcohol-soluble fraction was attained which had a neurotic effect upon mice which was four times as strong as the original poison. This fraction was electrophoretically non-uniform. Apparently it represented the poisonous principle in an impure condition.

8. The toxic proteins of the poison of the V. ammodytes can be selectively absorbed with filter paper. The antigen composition of aqueous solutions is therefore changed by filtering. The water-unsoluble precipitation of the poison of the V. ammodytes can be purified at the isoelectric point by precipitation from aqueous solution and shows the properties of a nontoxic protein. The Oudin test indicates the presence of three antigen components in the poison of the V. ammodytes which can be precipitated. In the raw poison, six or seven protein components could be recognized by their varying electrophoretic behavior.

If in neutralization experiments first the hemolytic and then the toxic effects are investigated up to end point determination, the authors obtained the same consumption of immunoserum. It can therefore be assumed that hemolytic and toxic effects are the properties of a single substance.

9. Determination of the molecular weight of the poison of V. ammodytes, its antidote and their aggregate was made in 0.15 K NaCl solution. Results: molecular weight for the poison
10. The reciprocal effect of the poison of V. ammodytes and the corresponding antidote were investigated by measuring light transparency and light diffraction. If opaqueness is represented graphically as a function of the ratio poison/antidote, a flocculate-type curve is obtained. It shows a maximum. This maximum corresponded to the neutralization point, as was demonstrated by measuring the hemolytic effect of the poison and the antitoxic effect of the antidote. The average molecular weight determined by light diffraction was 28,000 for the poison, 54,000 for the antidote and 120,000 to 3,100,000 for the poison/antidote aggregate depending upon the ratio.

11. Poison of the V. ammodytes was subjected to electrophoresis for two hours and the bands obtained were cut out and elutriated with salt solution. Coagulation experiments demonstrated coagulating and noncoagulating principles, whereby the coagulating fraction migrated to the anode, the noncoagulating to the cathode. The activity of the noncoagulating elutriate is dependent upon the concentration of prothrombin, proaccelerin and the factor VII complexes. The anticoagulant fraction showed antithromboplasten-like activity which was neutralized by thrombocyte and the Christman factor.

12. Electrophoresis of a ten percent solution of the crystallized toxin in physiological salt solution yielded 11 immunologically characterizable fractions in the cathode area and seven in the anode area. The electrophoretic investigation of snake serum showed that snake serum with antitoxic serum yielded six precipitation zones in the anode region near the start and two in the vicinity of the cathode.

13. The poison of the V. ammodytes was examined by chromatography and electrophoresis. It contained ten different fractions. Two of these behaved like glycoproteins. The others were proteins. None of the fractions showed oxidase activity or the characteristics of lipoproteins. Some proteins are denatured by lyophilization. Some fractions were not consistent in their immunological behavior — at least ten precipitation zones were observed on the electrophorogram. Two-dimensional electrophoresis yielded no additional separation. Sixteen different amino acids were identified. Methionin was present in plentiful degree.

14. The disulfide compounds contained in the poisons of orotalus adamanteus, vipera russelli and cobra were reduced with cystein. A reduction in phospholipase activity, the co-
agulant effect and toxicity were observed. Sulfhydryl compounds were found in the poison of the rattle snake and the vipera russelli after reduction. This was not the case with cobra poison. A marked toxin was obtained with \(^{35}\)S-marked cysteir.

15. Electrophoresis (Whatman No. 4 in 0.05 M veronal buffer of pH 6.6 at 200 volts and 16 mA, 16 hours) produced a complete separation of both enzymes. The phosphodiesterase obtained -- it migrates to the cathode -- contained no 5'-nucleotidase activity. Both enzymes retained the full activity for more than two years when stored under refrigeration.

16. The poisons of echis carinatus, Nafo oxiana, vipera lebetina (dried at room temperature) and those of V. lebetina and crotalus adamantus (lyophilized) were examined for their desoxyribonuclease activity. A non-specific reciprocal effect with desoxyribonucleic acids was observed for the lyophilized poisons. It was noticeable in the form of viscosity changes. This could not be observed in those poisons dried at room temperature. The desoxyribonuclease activity was greatest in the poison of the N. oxiana and the least in the poison of the V. lebetina; here lyophilized tests are more effective. The poisons of male snakes were more effective than those from females. The optimum pH range of enzyme activity was broad (5.0 to 9.0) in most of the poisons investigated, the single exception being rattle snake venom.

17. If a lethal dosage of the poison is used, only 67 percent mortality is obtained if 0.12 mg of heparin is added to the poison. With 0.6 mg of heparin only 34 percent mortality results. Incubation of poison and heparin prior to use reduces the detoxifying effect. Heparin is most effective when injected intravenously. If twice the lethal dosage is administered, no more effects of heparin can be observed.