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A Blood Anticoagulant Substance from Garlic(*Allium Sativum*)II.

Chemical Analysis and Studies on the Biochemical* and Pharmacological Effects

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ABSTRACT

G. E. as prepared in our laboratory is a non-volatile white substance, which is odorless and water soluble. Only in vivo does it have a hypotensive effect, while both in vivo and in vitro it has a hypo-calcemic effect. We determined the chemical analysis, toxicity, lethal dose, and the effect on isolated intestinal and auricular movements of rabbits of G. E. The sodium salt of G. E. contains 18.7% Phosphorus and 15.7% Sodium. It contains inositol and a small amount of sulfur and nitrogen.

The ratio of inositol: phosphorus: sodium is 1:6:6.7. Also G. E. may contain phytic acid and other mat erials which have not been identified. Toxicity tests of G. E. done on mice. The first symptoms of toxicity in mice began with irritability and unstable walking, which were followed by dyspnea and sluggish movements, and finally by coma. Mice LD 50 was 222 mg/kg

As the dose of G. E. was increased in successive injections in the rabbits, the rabbits died, when the total dose reached 100-200 mg%. Probably G. E. is not destroyed quickly nor excreted rapidly. The blood pressure in the rabbits continued to fall at each injection indicating no development of tachyphylaxis.

If 70 mg. of G. E. was injected intravenously, as one dose, the rabbit died with muscular hyperactivity. On post mortem examination, we found G. E. had a hypocalcemic effect. However if the calcium salt of G.E. was injected no muscular hyperactivity developed, but severe hypotension was observed. The hypocalcemic effect of G. E. is due to the combining of G. E. with the blood calcium and the muscular activity may be secondary to hypocalcemia.

The G. E. hypotensive effect in atropinized rabbits and in ganglionic blocked rabbits (Hexamethonium) was the same as the effect found in rabbits which had not been drugged. Epinephrine also did not change the hypotensive effect of G. E., G. E. itself showed no effect on the isolated intestinal and auricular movements of a rabbit as long as there were enough calcium ions in the solution. Hence we can not say that the hypotension of G. E. is due to vagus stimulation and or to paralysis of sympathetic nerve endings. The mechanism of the hypotensive effect of G.E. is not yet clear.

INTRODUCTION

Song et al (1963) isolated a blood anticoagulant substance from garlic (This substance is called Garlic Extract or G. E.). G. E. has not only an anticoagulant effect but also is toxic in rabbits. If one hundred milligrams of G. E. are injected into rabbits intravenously, they die.

In order to find the cause of G. E. toxicity, the lethal dose in mice, the change of serum calcium content, and the changes in blood pressure after administration of G. E. were investigated. The effect

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of G. E. on the heart and intestine were also investigated and a chemical analysis of G. E. was made.

MATERIALS AND METHODS

Sodium salt of G. E.: After the G. E. is dissolved in distilled water and neutralized with sodium hydroxide, alcohol is added, and the resultant is then refrigerated for 30 minutes. The precipitated sodium salt of G. E. is collected and dried.

Calcium salt of G. E.: The sodium salt of G. E. is dissolved in distilled water. Calcium chloride is added in sufficient amounts to precipitate all the G. E. The precipitated calcium salt of G. E. is collected and washed 3-4 times with distilled water and dried.

Hydrolysis of G. E.: 5.0 gm of calcium salt of G. E. was hydrolysed in 50 ml of 6 N hydrochloric acid under a reflux condensor for twenty hours. The hydrochloric acid was removed by the repeated adding and evaporating of alcohol. The residue was dissolved in ten ml. of water. then 5.0 ml of 10% calcium hydroxide solution were added, and the pH adjusted to neutral by adding sodium hydroxide and the mixture was then centrifuged.

The supernatant liquid was collected, alcohol added, and kept in a refrigerator, and if no crystals formed, it was centrifuged to precipitate the phosphate salt. The supernatant liquid conccentrated under reduced pressure. Alcohol was added and this was also kept in a refrigerator. The crystals were collected and recrystalized in a water alcohol mixture.

Hydrolysis of G. E. for quantitative analysis: One hundred mg of the sodium salt of G. E. was dissolved in one ml of 6N hydrochloric acid in a small tube and the tube was sealed and boiled at 110°C for 18~48 hours. The G. E. hydrolysate was transfered to a 100 ml volumetric flask, neutralized with sodium hydroxide and diluted to the mark with distilled water. Two ml of the solution were used for the quantitative analysis for inositol.

The determination of inositol was carried out according to Böhm's method(1954). Paper chromatography for inositol was carried out according to Hubscher's method(1957) except the ascending method in stead of the descending method was used.

The optical activity of G. E. solution was investigated by a Polarimeter.

Animals: White rabbits (body weight 1.5~2.0 kg), mice and frogs were used.

G. E. or Sodium salt of G. E. solution: G. E. or the sodium salt of G. E. is dissolved in distilled water or in 0.9% sodium chloride solution. It is neutralized with sodium hydroxide if necessary.

Calcium salt of G. E. solution: The calcium salt of G. E. is dissolved in distilled water adding hydrochloric acid. After it is completely dissolved, the solution is neutralized. When neutral, the calcium salt of G. E. forms a suspension.

For determination of the lethal dose, male mice weighing from 13 to 18 gms. were used. The G. E. was dissolved in physiological saline, and 0.25~0.5 ml of the solution injected into abdomen.

For investigation of the change in the calcium content of the serum: G. E. solution(2.5~5.0 ml) was injected into the ear vein of rabbits and then the calcium content of the serum was determined.

For investigation of the change in blood pressure: The carotid arterial blood pressure was registered on a smoked drum by means of a mercury manometer. The sodium or calcium salt of G. E. solution (2.0~5.0 ml) was injected into an ear vein in rabbits and the change of blood pressure was chymographed.

Atropine, Hexamethonium and Epinephrine were injected before or after the injection of the G. E. solution and the change of pressure was chymographed.

The method of investigation of intestinal and auricular movement of isolated intestine and heart was as follows:

The rabbits were sacrificed and immediately the intenstine was exposured by a midline incision, and about 30 cm of the small intestine was removed. The isolated intestine was soaked in Locke's solution and was cut into sections 2.0~2.5 cm long for chymography by the Magnus method.

Frog's heart movement was studied by the method of Engelman.

In studying auricular movements of the rabbit heart, the fasting rabbit was sacrificed and the heart

ever, after successive injections, the response was increased and when the total dose of G. E. reached to 100-200 mg, the rabbits died. It can be said that G. E. has a cumulative effect.

When a moderate dose of G. E. (30 mg) was injected, respiratory disturbances occured. When a large dose(about 70 mg) was injected at one time, the blood pressure fell to zero and the rabbits died.

The calcium salt of G. E. showed greater effect on the blood pressure than did G. E. itself. 15~30 mg. of calcium salt of G. E. killed the rabbits and its hypotensive effect as shown in Fig. 3 was very great.

B) Resonse of rabbits atropinized or treated with hexamethonium or epinephrine.

As is shown in Fig. 4 and 5. G. E. showed the same hypotensive effect in atropinized rabbits as in rabbits treated with hexamethonium. It was the same as in untreated rabbits. These drugs did not change the effect of G. E. on the blood pressure. Epinephrine also did not change the hypotensive effect of G. E.

C) The effect on auricular movement in isolated rabbit heart preparations.

As is shown in Fig. 6, 0.05% of G. E. inhibited the contraction of the auricle, but when calcium chloride was added drop by drop, the contraction returned to normal. The calcium salt of G. E. suspension had no effect on contraction.

D) The effect on the frog heart.

G. E. solution showed no effect on frog's heart movement. Even when a large dose of G. E. was injected into a frog's superficial abdominal vein, there was no effect on heart, nor did it kill the frogs.

E) The effect on intestinal movements of rabbits.

As is shown in Fig. 7, as the concentration of G. E. was elevated, it inhibited intestinal movements but when calcium chloride was added drop by drop, the. intestinal movements returned to normal.

The calcium salt of G. E. in the same concentration, had no effect on intestinal movement.

DISCUSSION

Hans Platenins(1935) isolated a volatile compound containing sulfur from Allium savitum. Another investigator, Abe(1938) precisely analysed the sulfur compound, and separated propylsulfide, diallyl disulfide and diallyl trisulfide.

Dittner(1929) stated that the allyl sulfide of the volatile compound has a antibacterial action and termed it allicin. It affected gram(+), gram(-) bacillae. He also confirmed that 1 mg of allicin was equivalent to 15 oxford units of penicillin. He analysed its chemical structure as $C_3H_5S(=O)SC_3H_5$ which is converted into $(C_3H_5S)_2$ by allicinase or by steam distillation.

Sgihara(1926) who separated a volatile substance from garlic demonstrated that it had a hypotensive effect and he thought that this hypotensive effect was due to depression of the central nervous system. This volatile substance was investigated by Kahwara and its chem icals tructure was found to $C_6H_{12}S_2$. This was found to be allicin by Cavalito, et al. (1944) 37years later, G. E. was separated from garlic in our laboratory. It has a hypotensive effect but is a non-volatile substance and so this material is quite different from the volatile substance separated by Sgihara.

When G. E. was injected intravenously into rabbits, it showed a hypotensive effect. The blood pressure fell abruptly with dyspnea and increasing muscular activity. G. E. has a hypocalcemic effect also, which is due to the powerful combination of G. E. with ionized calcium in blood. The muscular hyperactivity after G. E. injection might be due to a secondary hypocalcemia.

When the calcium salts of G. E. were administered by the same method, respiratory andmuscular hyperactivity were not observed.

In isolated intestine and heart muscle, G. E. inhibited the movement of the intestine and contraction of the heart but when calcium chloride was added, movement and contraction returned to normal. Calcium salts of G. E. showed no effect on heart contraction nor on intestinal movement. From these facts, it may be said that the effect of G. E. on the intestine and the heart is due to precipitation of calcium ions from the solution.

G. E. hypotensive effect in atropinized rabbits, and in hexamethonium ganglionic blocked rabbits is the same as in untreated rabbits. This is also

true with epinephrine. So we can not say that the hypotensive effect of G. E. is due to stimulation of the vagus endings or to paralysis of the sympathetic nerve endings. The mechanism of the hypotensive effect of G. E. is not yet clear.

As the total dose of G. E. on successive injections reached to 100–200 mg the rabbits died. G. E. may not be destroyed quickly nor excreted rapidly and the rabbits did not develop tachyphylaxis.

Concerning chemical analysis, G. E. contains inositol and phosphate, and the ratio is one to six, which means that G. E. may contain phytic acid. As the sodium salt of G. E. is not a pure material there is the possibility, that other substances are combined in it. Further investigation is necessary to find the active agent.

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