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A STUDY OF FIBRINOLYTIC ACTIVITY OF COELOMIC FLUID OF EARTHWORM (*PHERETIMA ASPERGILLUM*) FROM GUANGZHOU

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The results of disease caused by thrombus formation are serious. Streptokinase or urokinase (UK) is usually given by intravenous drip for fibrinolytic purpose clinically, but during the course several troublesome side-effect such as allergic reaction, disturbances of blood coagulation or fibrinolysis may occur. In addition both preparations are expensive. So search for some other safe effective fibrinolytic agents is still valuable.

In 1982 Mibara H., et al (1) extracted thrombolytic proteases from earthworm tissue, the proteases are stable for oral administration. Then we began to study earthworm from Guangzhou. In a previous paper(2), we reported the fibrinolytic activity had been found strongest in the coelomic fluid of earthworm (*Pheretima aspergillum*) from Guangzhou obtained by the Astrup's fibrin plate method(3). The fibrinolytic activity of 5 μ g of frozen dry crude powder of coelomic fluid was as effective as 1 IU of UK (Biochemical Pharmacy Factory of Tianjin) and showed fibrinolysis in fibrin plate heated at 85°C 30 min before using, but none by UK. The fibrinolytic activity in vivo had been studied with Umetsu's method(4). The frozen dry crude powder given to rats duodenum in a dose of 30 mg/kg body weight had decreased fibrin formation in a 59.01%, which was nearly to the effect of UK, 62.43% in a dose of 15000 IU/kg body weight, iv.

Earthworm is a rich source, easy for breeding and cheap, so we try isolate the effective composition from the coelomic fluid of earthworm, to study the influences of temperature and pH on its fibrinolytic activity and the toxicity of the preparation.

Earthworms (*Pheretima aspergillum*) provided partly by Biology Dept of University of Teachers of South China, were partly picked from rural area near Guangzhou city, the species had been identified. After isolation of coelomic fluid from earthworm, the fluid was frozen to dry crude powder contained 40.3% protein by Lowry's method, the yield was 1 mg/g wet(5), weight of earthworm.

Seven zones had been revealed after polyacrylamide gel electrophoresis(PAGE) of coelomic fluid stained with coomassie brilliant blue R250, the molecular weight of which were within the range of 67K to 12K as compared with standard proteins. There were two peaks in the elution

profile by filtration of coelomic fluid through Sephadex G75, three fractions had been collected, again each fraction had been divided into 6, 2 or 3 bands after PAGE, the separation was not ideal.

The fibrinolytic activity of the frozen dry powder of both coelomic fluid and fraction of Sephadex G75 filtrate stored at 5°C or -20°C were stable for at least over one year. Their fibrinolytic activity was similar over a temperature range from 27-37°C and a pH range from 4 to 10. No abnormal changes had been detected during acute toxicity test by 'upward or downward method'(6), with a dose of frozen dry crude powder up to 1 g/kg of body weight of mice fed by stomach tube or 400 mg/kg of body weight by ip.

Toxicity of some preparation of earth worm in China had been reported(7,*), e.g. an injective preparation in 450-720 fold of human therapeutic dose had been given to mice or guinea pig iv or ip, no death had occurred, the ID₅₀ was 95-115 g/kg. The LD₅₀ of water extract from visceral powder of earthworm was 38.5 g/kg. Canton earthdragon powder in a dose of 10 mg/kg body weight had been fed to rat by stomach tube, no toxic reaction had been observed.

The systemic effect by oral administration of enzyme is controversial, however some anti-inflammatory enzyme preparations are effective taken orally, such as bomelin, trypsin. Rothman et al (9) demonstrated an intestinal-pancreatic cycle of digestive enzyme. In our previous experiment the inhibitory effect of fibrin formation of frozen dry crude powder given to rat duodenum was obvious. But how much of the preparation had been 'absorbed', how long the effect can be sustained needed further investigation.

Roch et al (10), Honda et al (11) and cotuk et al (12) had reported that there were agglutinine, haemolysin, bacteriostatin and lysozyme activities in the coelomic fluid of earthworm (*Eisenta foetida*); how about these activities in the coelomic fluid of earthworm from Guangzhou, within the range of effective dose of frozen dry crude powder in the course of therapy; what would be the action on blood coagulation and other then fibrinolysis are also the question wanted to be answered.

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