Fibrinolytic Activity of the Endothelium

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The fibrinolytic activity of the endothelium of blood vessels in guinea pig and rat was demonstrated by histochemical and chemical procedures. The activity might be due to plasmin activator since the endothelium did not show any activity with heated fibrin. It was weak in the proximal aorta which has prevalence of atherosclerosis in rodent, and progressively stronger down the length of the aorta. Strongest activity was observed in the vein. The endothelium of rabbit, most susceptible animal to experimental atherosclerosis did not show any lytic activity against either bovine or rabbit fibrin. Role of fibrinolytic activity of the endothelium on atherosclerosis was discussed.

ROKITANSKY¹⁹⁾ proposed that deposition of fibrin, platelets or both may contribute to the development of atherosclerosis and proclaimed by DUGUID⁷⁾ and WOOLF²⁶⁾ who have found histological evidence of incorporation of fibrin into atheromatous lesions. ASTRUP²⁾ and COPLEY⁶⁾ stated an existence of a dynamic equilibrium state of continuous intravascular deposition counter-balanced by fibrin-dissolving activity. Recently, TODD²³⁾ and WARREN²⁵⁾ revealed that the endothelium of the blood vessels possessed fibrinolytic activity histologically. MESSER et al.¹⁷⁾ also revealed the evidence that the endothelium of veins possessed the fibrinolytic activity by using FOLIN-CIO-CALTEU color reaction. In this study, the evidence of different potency of fibrinolytic activity in the different levels of the aorta and the vein were obtained by histological and chemical methods.

MATERIALS AND METHODS

I. HISTOLOGICAL METHOD

Guinea pigs weighing about 500 grams of body weight, rats of about 300 grams and rabbits about 2 kilo grams body weight were used.

Thirty milligrams of bovine or rabbit fibrinogen (Nutritional Biochemicals Corporation) was dissolved in 1 ml of modified Michaelis buffer⁵⁾. Thrombin Topical was dissolved in 50 per cent glycerol to give a stock solution of 1000 units per ml. From this the working solution was prepared each time by dilution in Michaelis buffer pH 7.4 to contain 20 units per ml.

This fibrin membrane on cellophane was prepared according to the method of TODD²³⁾. A sheet of plate glass was accurately levelled by means of three screws and on it was placed wire frame 8 cm by 5.5 cm. A sheet of water wettable cellophane (Dupont 124PD), slightly larger than the frame, was soaked in Michaelis buffer and then squeegeed onto the glass. One ml. of fibrinogen solution and 0.14 ml of working thrombin solution were poured into the dish and

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TABLE I AVERAGE SCORE OF FIBRINOLYTIC ACTIVITY* OF THE ENDOTHELIUM EXAMINED BY THE TODD'S METHOD

Incubation time	No. of Animal	Arch.	Thoracic Prox.	Mid.	Dist.	Abdominal Prox.	Mid.	Dist.	V. cava
Guinea Pig									
4 hours	10	0.4	0.5	1.1	1.5	1.8	1.9	1.9	3.4
4 hours with streptokinase**	5	1.1	0.8	1.8	2.1	2.3	2.1	2.2	—
Rat									
1 hour	10	0.0	0.3	0.5	0.5	0.6	0.7	0.2	2.1
3 hours	10	1.3	1.9	2.1	2.4	2.7	1.6	1.7	3.0

* Graded from 0 to 4 plus, 0.5 representing minimal, and 4 representing prominent and diffuse lysis.

** 50 units of Streptokinase per 30 mg of bovine fibrinogen were added.

of one hour's incubation. Local difference of the lytic activity was not so prominent but the lower part of the thoracic and upper part of the abdominal aorta showed the strongest activity of the aortic sections.

The rabbit endothelium did not show any activity using either bovine or rabbit fibrin membrane with incubation at 37°C for as long as 24 hours. The vein of the rabbit was not examined.

II. CHEMICAL DETERMINATION OF THE FIBRINOLYTIC ACTIVITY

Chemical determination of fibrinolytic activity of guinea pigs revealed the highest activity in the vein as observed by histological methods. The released protein estimated as tyrosine was $0.64 \pm 0.39 \mu\text{g}/\text{mm}^2$ from endothelium of the thoracic aorta, $1.34 \pm 0.54 \mu\text{g}/\text{mm}^2$ from that of the abdominal aorta and $2.01 \pm 0.97 \mu\text{g}/\text{mm}^2$ from vena cava. Statistical analysis revealed a significant difference between the thoracic aorta and vein, and between the abdominal aorta and thoracic aorta ($p < 0.01$). A difference between the abdominal aorta and inferior vena cava was also significant ($0.05 < p < 0.10$). Surface fixed abdominal aorta still had rather higher activity as compared to other parts of the vessels. The difference of released tyrosine level between the tissue with fibrin and surface fixed tissue with fibrin was supposed to be the real fibrinolytic activity of the endothelium. This difference was $0.35 \mu\text{g}/\text{mm}^2$ for the thoracic aorta, $0.67 \mu\text{g}/\text{mm}^2$ for the abdominal and $1.47 \mu\text{g}/\text{mm}^2$ for the vena cava. These differences were statistically significant by the level of $p <$

TABLE II CHEMICAL DETERMINATION OF FIBRINOLYTIC ACTIVITY OF THE ENDOTHELIUM THE ACTIVITY WAS PRESENTED BY TYROSINE LEVEL (μG) PER UNIT AREA (mm^2) OF THE VESSEL. EACH GROUP WAS COMPOSED WITH 10 CASES.

Guinea Pig	
Thoracic Aorta	
Tissue with Fibrin	$0.64 \pm 0.39 \mu\text{g}/\text{mm}^2$
Surface Fixed Tissue w. Fibrin	0.29 ± 0.15
Tissue without Fibrin	0.25 ± 0.11
Abdominal Aorta	
Tissue with Fibrin	1.34 ± 0.54
Surface Fixed Tissue w. Fibrin	0.67 ± 0.40
Tissue without Fibrin	0.33 ± 0.35
Inferior Vena Cava	
Tissue with Fibrin	2.01 ± 0.97
Surface Fixed Tissue w. Fibrin	0.54 ± 0.34
Tissue without Fibrin	0.41 ± 0.19
Rat	
Thoracic Aorta	
Tissue with Fibrin	0.61 ± 0.29
Surface Fixed Tissue w. Fibrin	0.36 ± 0.23
Tissue without Fibrin	0.41 ± 0.12
Abdominal Aorta	
Tissue with Fibrin	1.12 ± 0.61
Surface Fixed Tissue w. Fibrin	0.31 ± 0.27
Tissue without Fibrin	0.72 ± 0.21
Inferior Vena Cava	
Tissue with Fibrin	2.60 ± 1.66
Surface Fixed Tissue w. Fibrin	0.93 ± 0.58
Tissue without Fibrin	0.54 ± 0.26

0.05.

Using the endothelium of rat aorta, the same results were obtained. That is: the released tyrosine level was $2.60 \pm 1.66 \mu\text{g}/\text{mm}^2$ for vena cava, $0.61 \pm 0.29 \mu\text{g}/\text{mm}^2$ for the thoracic aorta, and $1.12 \pm 0.61 \mu\text{g}/\text{mm}^2$ for the abdominal aorta. Statistically, a significant dif-

Fig. 3. Fibrinolytic activity of the endothelium estimated by released tyrosine from surface of the vessels.

The clear column denotes the value of released protein from vessel covered with fibrin membrane, the strip column, the value of endothelial surface fixed tissue with fibrin and the dark column represents the value from blood vessel covered with cellophane only.

ference between these three groups was observed ($p < 0.05$). The differences between tissue with fibrin and surface fixed tissue with fibrin of the thoracic, abdominal aorta and vena cava was $0.25 \mu\text{g}/\text{mm}^2$, $0.81 \mu\text{g}/\text{mm}^2$ and $1.67 \mu\text{g}/\text{mm}^2$ respectively. The difference was statistically significant in the abdominal aorta and the vein ($p < 0.05$) and suspected in the thoracic aorta ($0.05 < p < 0.1$).

Thus chemical analysis of fibrinolytic activity of the endothelium revealed higher activity of the abdominal aorta than that of the thoracic aorta, and the highest activity was in the vena cava in guinea pig and rat. (Table II and Fig. 3)

To examine the nature of the fibrinolytic activity of the endothelium, fibrin membrane was heated at 80°C in a moisture chamber for 45 minutes until the plasminogen in the fibrin was destroyed. Todd's technique was applied to this heated fibrin. Fibrinolytic activity of the endothelium was completely diminished with the heated fibrin membrane. This evidence may indicate that the endothelium releases plasmin activator into the vascular cavity.

DISCUSSION

Fibrinolytic activity of the endothelium showed species difference in rat, guinea pig

and rabbit against bovine fibrin. Histologically, rat endothelium seemed to have the strongest activity compared to the other kinds of animals. In rat, the lytic activity was first observed after a one hour's incubation. A three hour incubation for rat endothelium and four hour incubation for guinea pig endothelium showed almost the same extent of activity. Rabbit endothelium did not show any lytic activity against either bovine or rabbit fibrin by a four hour or longer incubation. WARREN²⁵⁾ has described the same species difference on the fibrinolytic activity of the endothelium. FRIEDMAN and BYERS⁹⁾ have revealed species differences of endothelial permeability, and described that the first few centimeters of the aorta of the young rabbit were found to be excessively permeable to both Evans blue and trypan blue but the young rat did not show such permeability. It is well known that rabbit was the most prevalent animal to induce experimental atherosclerosis, guinea pig is less susceptible to experimental atherosclerosis as compared to rabbit¹⁾, rat has rather more tolerance against experimental atherosclerosis. It is interesting that in those three species, there seems to be counter relation between fibrinolytic activity of the endothelium and susceptibility of the experimental atherosclerosis. ASTRUP⁸⁾ reported that higher activity of thromboplastin and low fibrinolytic activity in the endothelium and conversely, in the adventitia, higher fibrinolytic activity and lower thromboplastin activity were observed. The occurrence of species difference of thromboplastin activity in the endothelium will be suspected⁴⁾.

There is other well known evidence of the prevalence of atherosclerosis in the abdominal aorta in the human and dog, and the thoracic aorta in rabbit, guinea pig and rats. Many factors may concern such different distribution of atherosclerosis. It is possible that local mechanical factors may influence atherogenesis not only directly by an immediate action on arterial wall, but also indirectly by acting as a determinant of the arterial wall⁸⁾. On the other hand, the importance of intrinsic arterial characteristics in atherogenesis was demonstrated by transplantation experiments. Woyde