60. *Effect of Adrenaline and Noradrenaline on "Silicone-like Property" of Blood Vessels*

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The endothelial surface of blood vessels possesses a special characteristic like silicone.¹⁾ It prevents the adherence of blood platelets and leucocytes and preserves the fluidity of the blood.

Recently the authors¹⁾⁻⁵⁾ found that several amines, polypeptides and high molecular weight substances, capable of eliciting the host response of Landy and Shear,⁶⁾ induce swelling of endothelial cells resulting in the damaging of their "silicone-like property". Namely, the swollen endothelial cells exhibit stickiness to blood platelets and leucocytes and form white thrombi. At the same time the circulating platelets decrease in number and the blood clotting time shortens. It was also found that repetitious administration of these substances to various species of animals induces intimal arteriosclerosis. In regard to the problem of atherogenesis, the significance of adrenaline and noradrenaline has been already repeatedly discussed by many investigators.⁷⁾

The authors found that a small amount of adrenaline causes a transient damage to the "silicone-like property" of vascular endothelial cells, abrupt decrease in the number of circulating platelets and shortening of the blood coagulation time in rabbits and in men, while noradrenaline was found to be far weaker as compared to adrenaline.

Material and method. 63 male rabbits weighing 1.8–2.7 kg were used. In order to see the effect of adrenaline and noradrenaline on the "silicone-like property" of blood vessels, the mesenteric arteries and veins were observed under the microscope after surgical preparation under light anaesthesia in 12 male rabbits. Both surfaces of the mesenteric membrane were attached carefully on to the object glass. Using the oil immersion the blood vessel was magnified 1,200 times and using 16 mm super anscochrome color film PN1 the morphological changes were cinematographed. Adrenaline or noradrenaline, in the dose of 0.1, 1.0, 10.0, or 50 μ g per kg dissolved in 1 cc of saline, was administered intravenously into the vein of the ear. The blood specimens were also taken from the vein of the ear.

In the other experiment in animals without the above-mentioned operation, several different amounts of adrenaline were injected intravenously into the vein of ear after 12 hours of fasting, and before injection and 5 and 30 min, 1, 2, and 3 hours after injection blood specimens were taken from the vein of ear for blood counting and measurement of the whole blood coagulation time. The sampling of blood specimens was carefully performed from the vein of the ear utilizing the siliconized

syringe. Adrenaline was tested in the dose of $0.1 \mu\text{g/kg}$ in 10 animals, of $1.0 \mu\text{g/kg}$ in 8 animals, of $10 \mu\text{g/kg}$ in 11 animals, of $100 \mu\text{g/kg}$ in 2 animals.

The same experiment, using noradrenaline instead of adrenaline, was also performed on 20 rabbits. The dose of noradrenaline used was $0.1 \mu\text{g/kg}$ in 7 animals, $1 \mu\text{g/kg}$ in 5 animals, $10 \mu\text{g/kg}$ in 6 animals, and $100 \mu\text{g/kg}$ in 2 animals.

In 7 male adults aged 23–61 years old $0.1 \mu\text{g}$ per kg of adrenaline was administered intravenously and blood counting and measurement of the blood coagulation time were performed before and 5 and 30 min, 1 and 2 hours after injection. The blood specimen for measuring the blood coagulation time was sampled from the cubital vein with a siliconized syringe. In 5 men $0.1 \mu\text{g}$ per kg of noradrenaline was tested in the same way as adrenaline.

Olef's method was used for platelet counting and Sahli-Fonio's method was used for measuring the blood coagulation time. Adrenaline Sankyo and noradrenaline Levophed Winthrop were used.

Result. (1) In rabbits. As shown in the photographs, the endothelial cells of blood vessels were found to swell 1 or 2 minutes after the injection of $0.1 \mu\text{g}$, $1.0 \mu\text{g}$, or $10 \mu\text{g}$ per kg of adrenaline, and

1. A normal venule prior to injection of adrenaline. The endothelium is distinct.

2. The same venule seen in the figure 15 minutes after the injection of $1 \mu\text{g}$ per kg of adrenaline. Note: Adhesion of platelets and leucocytes

3. The same venule seen in the figure 45 minutes after the injection. Note: Disappearance of platelets and leucocytes

4. The same venule 15 minutes after the second injection of $1 \mu\text{g}$ per kg of adrenaline. Note: Adhesion of platelets and leucocytes

5. The same venule 50 minutes after the second injection. Note: Disappearance of platelets and leucocytes

3 or 5 minutes later stickiness of the swollen endothelial cells to platelets or leucocytes appeared and 5–20 minutes later the formation of platelet-thrombi or platelet-leucocyte thrombi became obvious. These vascular changes were observed relatively stronger in the vein, but were also observed in the artery. Also some fibrinous substance was found to be sticking on to the thrombi. The most interesting

evidence is the disappearance of these phenomena 20–85 minutes after the appearance of these phenomena. These phenomena produced by adrenaline were reproduced repeatedly over ten times in the same animal.

As shown in the figure, showing the average per cent change of blood count of each group of animals, the intravenous injection of 0.1, 1.0, and 10.0 μg per kg of adrenaline induced an abrupt decrease in the platelet count. The maximal decrease was encountered 5 or 60 minutes after injection, and in the case of larger doses of adrenaline, i.e. 10 μg per kg, 1 or 4 hours later the number began to increase after the initial decrease. By the administration of 100 μg per kg of adrenaline the initial decrease of the platelet was not observed but instead exhibited an abrupt increase.

The leucocyte count exhibited an increase and the erythrocyte count exhibited no appreciable change. In the case of 100 μg per kg of adrenaline the leucocyte count exhibited an initial decrease followed by a secondary increase.

The other striking evidence is the abrupt shorten-

ing of the blood coagulation time accompanying the decrease of platelet count. The maximal shortening was observed in the first 5 or 30 minutes by 0.1 μg per kg of adrenaline and the per cent change from the control was $-24\% \pm 19.2\%$ for beginning time and $-20\% \pm 12.1\%$

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for completion, and 2 or 3 hours after the administration of adrenaline the blood coagulation time returned to normal accompanied by the recovery of the platelet count. The shortening of the blood coagulation time in the case of $0.1-1 \mu\text{g}$ per kg of adrenaline is striking and similar, while it was not so striking in the case of $10 \mu\text{g}$ per kg.

Noradrenaline exhibited no or a minimal initial decrease of platelet count as well as slight shortening of the blood coagulation time by the administration of 0.1, 1.0, $10.0 \mu\text{g}$ per kg as shown in Table I. In accordance with these evidences the direct observation of blood vessels revealed no or a minimal change of endothelial cells after the injection of noradrenaline and no stickiness of endothelial cells to platelets and leucocytes was induced even by successive injections with 1 or $50 \mu\text{g}$ per kg of noradrenaline.

Table I. Effect of adrenaline and noradrenaline on platelet count and blood coagulation time in rabbit

		Before	5 m	30 m	1 h	2 h	4 h
Adrenaline $0.1 \mu\text{g/kg}$ (10 cases)	Platelet count	472,000 $\pm 70,000$	396,000 $\pm 66,000$	330,000 $\pm 66,000$	307,000 $\pm 67,000$	363,000 $\pm 21,000$	430,000 $\pm 55,000$
	%	100.0	83.7 ± 13.9	70.1 ± 14.0	64.9 ± 14.2	76.5 ± 4.5	91.3 ± 11.7
	Coagulation time Begin, %	$4'05'' \pm 10''$ 100.0	$3'07'' \pm 47''$ 76.3 ± 19.2	$3'31'' \pm 43''$ 86.5 ± 17.6	$3'58'' \pm 15''$ 97.0 ± 6.0	$4'11'' \pm 12''$ 102.5 ± 5.0	$4'18'' \pm 5''$ 105.0 ± 2.0
	End %	$6'45'' \pm 1'30''$ 100.0	$5'31'' \pm 49''$ 82.3 ± 12.1	$5'56'' \pm 1'06''$ 88.3 ± 16.3	$5'33'' \pm 29''$ 82.8 ± 7.2	$6'49'' \pm 1'20''$ 100.3 ± 19.8	$7'15'' \pm 1'08''$ 109.5 ± 16.7
Adrenaline $1.0 \mu\text{g/kg}$ (8 cases)	Platelet count	473,000 $\pm 153,000$	348,000 $\pm 44,000$	334,000 $\pm 75,000$	322,000 $\pm 52,000$	449,000 $\pm 91,000$	393,000 $\pm 133,000$
	%	100.0	73.6 ± 9.3	70.6 ± 15.8	68.0 ± 11.0	95.0 ± 19.3	83.0 ± 28.1
Adrenaline $10.0 \mu\text{g/kg}$ (11 cases)	Platelet count	527,000 $\pm 121,000$	355,000 $\pm 158,000$	355,000 $\pm 113,000$	435,000 $\pm 117,000$	402,000 $\pm 47,000$	440,000 $\pm 128,000$
	%	100.0	74.6 ± 30.0	74.6 ± 21.5	91.6 ± 22.2	85.0 ± 9.0	92.7 ± 24.2
Nor- adrenaline $0.1 \mu\text{g/kg}$ (7 cases)	Platelet count	440,000 $\pm 68,000$	418,000 $\pm 47,000$	436,000 $\pm 59,000$	449,000 $\pm 40,000$	471,000 $\pm 39,000$	484,000 $\pm 85,000$
	%	100.0	95.4 ± 10.7	99.4 ± 13.4	101.7 ± 9.2	106.7 ± 9.0	109.9 ± 19.3
	Coagulation time Begin, %	$4'20'' \pm 1'29''$ 100.0	$3'36'' \pm 23''$ 82.5 ± 8.7	$3'48'' \pm 58''$ 86.2 ± 22.1	$3'54'' \pm 47''$ 90.0 ± 18.2	$4'00'' \pm 25''$ 92.0 ± 9.7	$4'10'' \pm 11''$ 95.6 ± 4.2
	End %	$7'23'' \pm 1'48''$ 100.0	$6'10'' \pm 45''$ 83.0 ± 10.2	$6'19'' \pm 46''$ 83.6 ± 7.8	$6'32'' \pm 51''$ 88.8 ± 11.4	$6'32'' \pm 25''$ 88.8 ± 5.6	$6'26'' \pm 51''$ 88.0 ± 11.5
Nor- adrenaline $1.0 \mu\text{g/kg}$ (5 cases)	Platelet count	541,000 $\pm 83,000$	481,000 $\pm 62,000$	471,000 $\pm 28,000$	465,000 $\pm 61,000$	595,000 $\pm 127,000$	530,000 $\pm 113,000$
	%	100.0	89.4 ± 11.5	88.6 ± 5.1	86.2 ± 11.4	110.4 ± 23.4	98.2 ± 20.9
Nor- adrenaline $10.0 \mu\text{g/kg}$ (6 cases)	Platelet count	489,000 $\pm 182,000$	486,000 $\pm 21,000$	518,000 $\pm 81,000$	465,000 $\pm 107,000$	542,000 $\pm 145,000$	543,000 $\pm 111,000$
	%	100.0	99.5 ± 4.2	105.8 ± 16.6	94.6 ± 21.8	111.0 ± 29.6	111.3 ± 22.6

(2) In men. The administration of $0.1 \mu\text{g}$ per kg of adrenaline exhibited often slight elevation of the heart rate and of the blood pressure, but two cases remained without these changes. On the other hand all 7 cases exhibited regularly and definitely an abrupt decrease in the platelet count and shortening of the blood coagulation time, even in the cases in which there was found no significant elevation of

blood pressure and of the heart rate. The maximal decrease of platelet count and of the shortening of the blood coagulation time appeared 5 min after the administration and 2–3 hours thereafter these changes returned slowly to normal.

The injection of 0.1 μ g per kg of noradrenaline was performed on 5 men. In 2 men there was found no change of blood pressure and heart rate, but in the other 3 men the heart rate and the blood pressure exhibited a slight increase. The platelet count exhibited an abrupt but extremely slight decrease and the blood coagulation time exhibited an abrupt and slight shortening as in the case of adrenaline.

Table II. Effect of adrenaline on the platelet count and blood coagulation time in man

Adrenaline	Before	5 m	30 m	1 h	2 h
Platelet count	343,000 $\pm$ 22,000	237,000 $\pm$ 17,000	283,000 $\pm$ 12,000	290,000 $\pm$ 16,000	319,000 $\pm$ 13,000
%	100.0	69.3 $\pm$ 7.2	82.6 $\pm$ 3.7	84.4 $\pm$ 5.4	93.0 $\pm$ 4.2
Coagulation time, Begin	13'04" $\pm$ 37"	8'13" $\pm$ 36"	10'17" $\pm$ 23"	9'50" $\pm$ 26"	12'04" $\pm$ 40"
%	100.0	66.3 $\pm$ 7.3	81.0 $\pm$ 3.8	78.0 $\pm$ 4.5	93.3 $\pm$ 5.5
End	22'04" $\pm$ 41"	15'00" $\pm$ 1'12"	19'25" $\pm$ 1'24"	19'12" $\pm$ 23"	20'12" $\pm$ 46"
%	100.0	70.7 $\pm$ 6.8	89.0 $\pm$ 6.0	88.6 $\pm$ 2.1	97.3 $\pm$ 3.8

Effect of noradrenaline on the platelet count and blood coagulation time

Platelet count	236,000 $\pm$ 11,000	216,000 $\pm$ 3,000	230,000 $\pm$ 0	238,000 $\pm$ 17,000	225,000 $\pm$ 3,000
%	100.0	92.5 $\pm$ 1.8	97.6 $\pm$ 0.0	103.0 $\pm$ 7.1	94.3 $\pm$ 1.5
Coagulation time, Begin	8'53" $\pm$ 34"	7'30" $\pm$ 30"	9'00" $\pm$ 24"	9'10" $\pm$ 22"	8'40" $\pm$ 24"
%	100.0	84.4 $\pm$ 4.1	101.3 $\pm$ 4.4	103.2 $\pm$ 4.4	97.6 $\pm$ 8.4
End	17'45" $\pm$ 43"	15'30" $\pm$ 21"	17'04" $\pm$ 31"	18'05" $\pm$ 44"	18'00" $\pm$ 1'06"
%	100.0	89.2 $\pm$ 8.2	96.7 $\pm$ 3.2	104.2 $\pm$ 4.2	101.4 $\pm$ 6.1

Discussion. The most striking finding in this experiment is the transient damage of the "silicone-like property" of endothelial cells of blood vessels by a very small amount of adrenaline, i.e. a transient appearance of swelling of endothelial cells, stickiness of these cells to platelets and leucocytes and formation of white thrombi and the rapid disappearance of these changes and repetitious reproducibility of these changes by successive injections in animals. Also the abrupt and striking decrease in the platelet count and the shortening of the blood coagulation time in man and animal by such a small amount of adrenaline have not yet been described in literature.

The authors found that substances capable of eliciting the above-mentioned phenomena, produce intimal arteriosclerosis experimentally in animals,¹⁾⁻⁵⁾ and in accordance with these facts it is well known that the repetitious administration of a smaller amount of adrenaline produces intimal arteriosclerosis⁸⁾⁻¹¹⁾ instead of Mönckeberg's sclerosis.

On the other hand the relative ineffectiveness of noradrenaline in injuring the "silicone-like property" of the blood vessels is also a striking evidence. These findings may suggest an important difference in the atherogenic property of various stresses which induce a hypersecretion of both catecholamines in a different proportion according to

the difference of the type of stress.

In addition it is also well known¹²⁾ that catecholamines liberate the plasminogen activator in the blood. This effect may counteract the formation of intimal arteriosclerosis, so that there might be considered quite complicated factors.

The injurious effect itself on the "silicone-like property" by any of the arteriosclerogenic substances may be considered as the "germ" of intimal arteriosclerosis and such a powerful effect of a small amount of adrenaline of physiological significance shown in this experiment may suggest its important role in the formation of intimal arteriosclerosis including atherosclerosis especially under the coexistence of factors injuring the above-mentioned property of endothelial cells and disturbing the recovery process of injured endothelial cells.

Summary. Using the direct observation technique in situ of mesenteric blood vessels of rabbits under the microscope, the intravenous administration of a small amount of adrenaline in a dose like 0.1, 1.0, 10.0 μg per kg was found to induce a transient damage to the "silicone-like property" of blood vessels, i.e. a transient swelling and stickiness of endothelial cells to platelets and leucocytes often forming platelet or platelet-leucocyte thrombi. Such evidences were repeatedly produced in the same animal by successive administration and were cinematographed. In accordance with these evidences the number of circulating platelets decreases immediately, i.e. 5–30 minutes after the injection of 0.1–1.0 μg per kg of adrenaline and at the same time the blood coagulation time was shortened.

In man the intravenous injection of 0.1 μg per kg of adrenaline was found to induce similar changes as in rabbits, i.e. immediate and transient decrease in the number of circulating platelets and abrupt and transient shortening of the blood coagulation time.

On the other hand the intravenous administration of noradrenaline in the same dose in man and rabbit was found to be less effective in regard to the above-mentioned effects.

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