Studies on the Acid Polysaccharides in Blood Vessel Walls (3)

INFLUENCE OF ACID POLYSACCHARIDES ON CAPILLARY FRAGILITY AND CAPILLARY PERMEABILITY IN EXPERIMENTAL HEMORRHAGIC DIATHESIS

By

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The author studied the influence of "hi" acid and "cho" sulfate on capillary fragility and permeability of guinea pigs with experimental hemorrhagic diathesis (scurvy, thrombopenic purpura, vascular purpura). Capillary permeability is determined by the concentration of T-1824 in the plasma, and capillary fragility by the Borbély negative pressure method. Capillary permeability and fragility of normal male rats did not increase or decrease by administration of "hi" acid or "cho" sulfate. The increase of capillary permeability and fragility in experimental scorbutic guinea pigs was ameliorated by administration of Vitamin C or "hi" acid. "hi" acid seemed to inhibit the increase of capillary fragility and permeability in experimental thrombopenic purpuric guinea pigs. Increased capillary fragility of experimental vascular purpura was decreased by administration of "hi" acid. It seemed that "cho" sulfate did not ameliorate the increase of capillary permeability and fragility in the experimental hemorrhagic diathesis. Capillary fragility and permeability were not always parallel each others. In experimental vascular purpura, when the capillary fragility began to increase, the capillary permeability decreased. The contents of acid polysaccharides in blood vessel walls in animals with experimental hemorrhagic diathesis were approximately the same as in normal animals.

AS STATED in Parts I and II, blood vessel walls contain hyaluronic acid (abbreviated as "hi" acid) and chondroitin sulphuric acid (abbreviated as "cho" sulfate). In regard to the bio-chemical nature and physiological function of "hi" acid and "cho" sulfate, it is said that "hi" acid seems to serve to maintain tissue structure as inter-cellular cement, and by its high viscosity protects against the invasion of pathological organisms⁵⁷⁾. And it is believed that it is involved in water metabolism and in the exchange of many kinds of metabolites, because of its great affinity for water⁵⁰⁾. In the connective tissues of patients with hypothyroidism the hyaluronic acid is of high molecular value²⁾. The synovial fluid in rheumatoid arthritis is of

much lower viscosity than normal⁴⁵⁾. This phenomenon indicates that the disease is characterized by a deficiency in biosynthesis or a failure to complete the polymerization of hyaluronate. Recently it has been reported that oral administration of glucuronic acid enhances the production of "hi" acid in vivo⁵⁷⁾. Mayer and Chaffee found hyaluronate in some malignant tumors³⁸⁾. As stated by Asobe-Hancen, hyaluronic acid seems to be produced by mast cells³⁾. Probably "cho" sulfate contributes to the elasticity of connective tissue⁵⁷⁾. The oral administration of "cho" sulfate is effective in idiopathic headache in humans¹¹⁾. Oshima stated that oral administration of "cho" sulfate relieved the pain of migraine, neuralgia, myalgia, chronic

arthritis, and peri-arthritis⁴⁰⁾⁴¹⁾. Oral administration of "cho" sulfate tends to weaken cholinesterase activity in the blood⁴¹⁾, to have a diuretic effect⁴⁰⁾, to increase general sulfate excretion in urine, and to decrease neutral sulfate excretion. And "cho" sulfate increases the permeability of tissues⁴⁰⁾. Wolf from stated that "cho" sulfate has an inhibitory effect on blood coagulation⁵⁴⁾. "cho" sulfate is probably produced by fibroblasts⁶⁾. In regard to the action of acid polysaccharides on blood vessel walls, Oshima reported that "cho" sulfate has a vasodilating action⁴¹⁾, and Nomura said it decreases the blood vessels' permeability³⁹⁾. Rappert and Cella reported that "hi" acid is related to the fragility of capillary blood vessels⁴⁶⁾.

The author investigated the influence of "hi" acid and "cho" sulfate on capillary fragility and blood vessel permeability in animals with experimental hemorrhagic diathesis and also investigated histochemically the acid polysaccharides in the blood vessels of the animals.

EXPERIMENTAL MATERIAL AND METHODS

Guinea pig: male guinea pigs (weighing about 300 gm) were employed as test animals.

Examination of T-1824 concentration in blood:

The author dissolved 4 mg T-1824 (Evans blue) in 0.4 ml of physiological salt solution and

injected it in the jugular vein, and removed 0.7 ml of blood from the other side in a syringe moistened with heparin after 5 minutes, 30 minutes, 60 minutes, 90 minutes and 120 minutes. The samples of blood were centrifuged for 30 minutes at 3000 r.p.m., and to each 0.1 ml of plasma 2.9 ml of distilled water was added. The optical densities were determined at 620 m μ by the electric photometer. The author considered as 100 per cent the concentration of T-1824 in the plasma after 5 minutes. As shown in Fig. 1, the relation between the concentration of T-1824 and the optical density made a straight line.

Examination of capillary fragility:

The author measured the capillary fragility by the Borbely negative pressure method. Namely, the author used Okubo's petechiometer with a cupping-plate of 1 cm inside diameter, and the author determined capillary resistance at the minimum mercury pressure causing bleeding from capillaries with suction for one minute. Preparatory to this, the author cut off the hairs around the chest and abdomen of the guinea pig with scissors, fit the cupping-plate close to the body and protected mechanical injury for it by smearing with white vaseline. The author adopted petechiae in the central part, ignoring the petechiae around the cupping-plate. The author did not use any depilatory which might influence capillary fragility.

Experimental scurvy:

Twenty five guinea pigs were fed in wire net cages to prevent corprophagia. Each guinea pig was fed with 80 gm of Tofukasu, the

Fig. 1. The relationship between concentration of T-1824 and optical density.

matsukaram mixture salt 6 gm, Ebios (yeast) 0.1 gm, Vitamin B₆ 50 r. and liver oil 0.25 drops daily. Animals were divided into four groups as follows.

Scorbutic diet	10 cases
Scorbutic diet+subcutaneous Vitamin C	5 cases
Scorbutic diet+subcutaneous "hi" acid	5 cases
Scorbutic diet+subcutaneous "cho" sulfate	5 cases

Experimental thrombopenic purpura:

In order to obtain guinea pig thrombocytes, blood was removed from the carotid artery by a syringe containing 4 ml of Watabiki's solution per 10–15 ml of blood. (10–15 ml of blood be obtained from each guinea pig. In order to sensitize one rabbit, thrombocytes isolated from 30 ml of guinea pig blood are needed; it is more efficient to use fresh blood.) 50 ml of guinea pig's blood was centrifuged for 5 minutes at 2000 r.p.m. and the light yellow, slightly turbid supernatant was mixed with physiological salt solution and again centrifuged for 30 minutes at 3000 r.p.m. The precipitate was observed under a microscope and it was verified that it contained scarcely any erythrocytes or leucocytes and it consisted almost completely of thrombocytes. It was then, suspended in about 20 ml of salt solution, and this solutiso was used as the antigen for one experiment. The author injected the fresh thrombocyte suspension into a rabbit's ear vein 6 times at 2 to 3 day intervals. 10 days after the last injection, the author removed blood. Serum was warmed for 30 minutes at 56°C for inactivation, phenol was added to make a 0.5 % solution which was then placed in an ice box. The agglutination of this serum was positive at 1/1424 for thrombocytes and 1/128 for erythrocytes. T-1824 was injected into guinea pigs' jugular veins, and blood was removed from the jugular vein of the other side 5 minutes after the injection. At the same time they were given subcutaneous injections of 1.0 ml of anti-thrombocyte serum. Animals were divided into three groups as follows:

Group 1: Unteated antithrombocytopenic purpura	5 cases
Group 2: Antithrombocytopenic purpura treated by 5 mg "cho" sulfate	5 cases
Group 3: Antithrombocytopenic purpura treated by 10 mg "cho" sulfate	5 cases

Experimental vascular purpura:

Endothel cell from the aorta of five guinea pigs was suspended in 20 ml of physiological salt solution. The suspension was injected into rabbits' ear veins 7 times at intervals of 4 days. The author removed blood from the rabbits' hearts after being sure of an increase in agglutination of the endothel cell 10 days from the last injection of the suspension. Serum was made inactive by heating at 56°C for 30 minutes, 0.5 % phenol added to it and kept in an ice box. The agglutination endotheliol cells by this serum was positive up to 1/1424 dilution and of thrombocytes up to 1/128 dilution. The experimental animals were divided into 3 groups:

1. Vascular purpura untreated	5 cases
2. Vascular purpura-5mg "hi" acid	5 cases
3. Vascular purpura-10 mg "cho" sulfate	5 cases

The author investigated the effect on normal animals of the administration of "hi" acid and "cho" sulfate, before observing their effect in animals with experimental hemorrhagic diathesis. Animals were divided into 6 groups as followi:

untreated group	10 cases
group given 5 mg "hi" acid	5 cases
group given 10 mg "cho" sulfate	5 cases
group given hyaluronidase	5 cases
group given inactive hyaluronidase	5 cases
group given physiologic salt solution	5 cases

RESULTS

- 1) Influence of hyaluronidase and acid polysaccharides on blood vessel permeability in the normal guinea pigs

In the untreated group, as shown in Table 4, the T-1824 density in the blood was 84.83 % at 30 minutes, 67.47 % at 60 minutes, and 62.82 %

TABLE I T-1824 DENSITY IN THE PLASMA.

	Untreated group (10 cases)	Subcutaneous injection of "hi" acid 5mg. daily for 5 days (5 cases)	Subcutaneous injection of "cho" sulfate 10mg. daily for 5 days (5 cases)
30 minutes	84.83 ± 6.46	82.63 ± 5.32	86.32 ± 5.38
60 minutes	67.47 ± 2.63	68.72 ± 4.66	69.72 ± 4.76
90 minutes	62.82 ± 3.67	63.72 ± 6.73	63.42 ± 5.32

at 90 minutes. In the two groups receiving subcutaneous injections of "hi" acid 5 mg daily or "cho" sulfate 10 mg daily for five days, as shown in Table I, there was no significant difference in comparison with the untreated group. After intravenous injection of 5 mg of "hi" acid or 10 mg of "cho" sulfate, as shown in Table II, it only moved around the standard area. In the hyaluronidase administered group, after 5 minutes of intravenous injection of 0.41 ml (4mg) of T-1824, 0.7 ml of blood was removed from the jugular vein, and this was immediately followed by injection of hyaluronidase (Motida Co. Ltd.) 20,000 V.U.M. dissolved in 0.5 ml of physiologic salt solution into the opposite jugular vein, and the density of T-1824 in the blood was observed successively. As controls, one group was injected with hyaluronidase which had been inactivated by 30 minutes heating at 70°C and the other group was injected with 0.5 ml of physiological salt solution. As shown in Table III, after 30 minutes the hyaluronidase administered group showed a slight increase in permeability, but after 60 and 90 minutes the degree of permeability was almost

in "the standard area". After intravenous injection of T-1824, one case received repeatedly intravenous injection of 20,000 V.U.M. of hyaluronidase after 5 and 30 minutes, and another case received repeatedly intravenous injections of 20,000 V.U.M. of hyaluronidase at 5 minutes and 0.5 ml of physiological salt solution at 30 minutes. No changes of permeability were found in either animal (Table IV).

- 2) Influence on the permeability and fragility of precapillary blood vessels after administration of acid polysaccharides in experimental scurvy.

When guinea pigs were fed with a scorbutic diet, they had good appetite at first. This condition continued about two weeks (so-called incubation period). Then, they lost appetite, developed depressive state, inaction, and gradual weight loss. Their hair lost its luster and fell out. They had diarrhea and soiled their abdomens, hips, and hind legs with excrement. Their activity became slower, they became increasingly emaciated and finally died. As shown

TABLE II T-1824 DENSITY IN THE PLASMA AFTER INTRAVENOUS OF "HI" ACID 5 MG OR "CHO" SULFATE 10 MG.

	intravenous injection of "hi" acid 5mg. (one case)	intravenous injection of "cho" sulfate 10mg. (one case)
30 minutes	81.03	87.62
60 minutes	70.68	71.87
90 minutes	65.51	64.06

TABLE III T-1824 DENSITY IN THE PLASMA AFTER THE INTRAVENOUS INJECTION OF HYALURONIDASE 20,000 V. U. M. (33,000 V. U. M/NMG)

	Normal	Hyaluronidase 20,000 V.U.M.	Hyaluronidase (inactivated)	Physiologic salt solution (0.5 ml.)
30 minutes	84.83 ± 0.46	77.19 ± 8.35	81.45 ± 5.38	84.25 ± 5.23
60 minutes	67.47 ± 2.63	65.18 ± 5.34	67.23 ± 4.46	70.87 ± 5.65
90 minutes	62.82 ± 3.67	60.46 ± 5.37	61.38 ± 5.56	61.41 ± 4.68

TABLE IV T-1824 DENSITY IN THE BLOOD PLASMA AFTER INTRAVENOUS INJECTION OF HYALURONIDASE 20,000 V. U. M. TWO TIMES.

	After intravenous injection of T-1824 one case had intravenous injection two times each of 20,000 VUM of hyaluronidase at 5 minutes, at 30 minutes.	After intravenous injection of T-1824 one case had intravenous injection 20,000 VUM of hyaluronidase at 5 minutes and 0.5ml of physiologic salt solution at 30 minutes.
30 minutes	78.16	80.16
60 minutes	64.22	63.22
90 minutes	57.86	59.38

in Table V, macroscopically, petechiae are seen specially in the lungs, mesentery, kidney, adrenal glands and peritoneum, and some of them had bloody ascites.

Capillary permeability: Capillary permeability on the 22nd day of experimental scurvy showed a distinct increase over normal cases after 30 minutes, and after 60 minutes they showed scarcely any increase in permeability, and after 90 minutes they showed a distinct increase in permeability again (Table VI). The author examined capillary permeability in two scorbutic guinea pig groups of which one group received daily subcutaneous injections of Vitamin C 250 mg for 5 days from the 16th day of the experiment, and the other group one intravenous injection of Vitamin C 250 mg on the 22nd day of the experiment. In the first group the guinea pigs recovered from scorbutic states clinically, and al-

most completely regained their health and their capillary permeability returned to normal, but in the second group capillary permeability was not healed. In the group which received daily subcutaneous injections of 5 mg of "hi" acid for 5 days from the 16th day of experiment, there was a slight decrease in permeability as compared with the untreated scorbutic group, but it was greater than that of the normal group. And the group which received daily subcutaneous of "cho" sulfate 10 mg for 5 days from the 16th day of experiment showed an obvious increase of permeability over the normal group and almost the same degree of permeability as the untreated scorbutic group did.

Capillary fragility: In normal guinea pigs it required a negative pressure of more than 300 mg on the skin of both the chest and abdomen to cause capillary fragility. As shown in

TABLE V WEIGHT LOSS AND AUTOPSY FINDINGS IN EXPERIMENTAL SCORBUTIC ANIMALS.

No of guinea pig	sex	days of survival	weight before exp.	weight after exp.	gain or lose in weight	Autopsy findings	Food
1	0	27	330 gm.	250 gm.	-80 gm.	Hemorrhagic diarrhea. Bleeding in lung and intestine. Depilation in abdomen.	Tofu-Kasu 80.0gm Ebios 0.1 Vitamin B6 50r Liver oil 0.25 drop
2	0	15	330	200	-130	Hemorrhagic diarrhea. Bleeding in adrenal gland, intestine.	Matsu Karam Mixture salt 1gm
3	0	18	370	310	-60	Intestinal bleeding.	Na Cl - 17.3 Na H ₂ PO ₄ ·H 20 - 34.7 K ₂ HPO ₄ -95.4 CaH ₄ (PO ₄) ₂ ·H ₂ O -54.0 Fe-Lactate-11.8 Ca-Lactate-139.0 Mg SO ₄ -26.6 KI - 1.0
4	0	24	320	270	-50	Bleeding in adrenal gland, u. intestine.	
5	0	22	290	230	-60	Depilation. Intestine bleeding. Hemorrhagic diarrhea.	
6	0	18	320	290	-30	Mesenteric lymphgland bleeding. Adrenal gland bleeding.	
7	0	17	265	210	-55	Subcutaneous tissue edema. Bleeding in intestine, lung, adrenal gland.	
8	0	18	410	350	-60	Bleeding in adrenal gland, intestine mesenteries.	
9	0	16	460	360	-100	Depilation. Adrenal gland bleeding.	
10	0	12	330	270	-60	Lung bleeding.	
11	0	25	300	250	-50	Depilation. Adrenal gland bleeding.	
12	0	20	400	350	-50	Mesenteric lymph gland bleeding. Adrenal gland bleeding.	
13	0	27	300	320	+20	(-)	To the above food Vitamin C powder 100mg. per 100gm. of body weight was added.
14	0	23	285	310	+25	(-)	
15	0	22	300	310	+10	(-)	

TABLE VI CAPILLARY PERMEABILITY IN EXPERIMENTAL SCURVY.

	Normal	Scurvy	Daily subcutaneous injection of Vitamin C 250mg. for 5 days.	Daily subcutaneous injection of "hi" acid 5mg. for 5 days	One intravenous injection of Vitamin C 250mg.	Daily subcutaneous injection of "cho" sulfate 10mg. for 5 days
30 minutes	84.83±6.46	74.69±5.13	83.43±5.78	79.83±6.89	75.83±6.78	75.32±6.78
60 minutes	67.47±2.63	64.06±4.32	66.45±3.27	64.78±6.45	68.95±5.92	63.45±4.43
90 minutes	62.82±3.67	52.73±5.20	60.35±4.57	56.67±5.95	50.48±6.63	53.56±4.45

Table VII, in the scorbutic animals which had lived more than 20 days 30 to 50 mmHg negative pressure was sufficient (the numbers in parentheses represent numbers of petechiae), that is, the scorbutic guinea pigs had increased capillary fragility. Capillary fragility in the experimental scorbutic guinea pig developed at 250 mmHg of negative pressure on the chest on the 12th day of experimental scurvy, while their general condition seemed still healthy (incubation period). Then, with the aggravation in their clinical condition, there was a rapid increase in capillary fragility. In considering the effect of subcutaneous injections of "hi" acid 5 mg, "cho" sulfate 10 mg, or Vitamin C 100 mg, as shown in Table VIII, in the case of "hi" acid there was an increase in capillary resistance for 3 to 18 hours, but after

24 hours it returned to its former degree of fragility. With "cho" sulfate there was no change in capillary resistance, and with Vitamin C, capillary resistance was 150 mmHg at 12 hours after the injection, and after the second subcutaneous injection of Vitamin C 100 mg capillary resistance in scorbutic guinea pigs became 250 mmHg at 48 hours after the first injection.

Histo-chemical studies of acid polysaccharides in the blood vessel wall, using Lissens stain and P.A.S. stain, revealed no differences between the experimental scurvy group and normal guinea pigs.

- 3) Influence of acid polysaccharides on capillary fragility and permeability in experimental thrombocytopenic purpura.

TABLE VII CAPILLARY PERMEABILITY IN EXPERIMENTAL SCORBUTIC ANIMALS.

Guinea pig No.	Sex	Days of survival	Weight before the Exp.	Weight after the exp.	Gain or lose weight	Autopsy findings	Capillary Resistance (negative pressure)
18	0	22	260 gm.	200 gm	-60 gm	Adrenal gland bleeding.	30 mm Hg (3)
21	0	23	330	220	-110	Intestinal bleeding.	30 mm Hg (6)
22	0	19	250	210	-40	" "	—————
23	0	19	340	270	-70	Hemorrhagic diarrhea	30 mm Hg (10)
24	0	25	250	190	-60	Intestinal bleeding.	30 mm Hg (6)
25	0	20	260	185	-75	Intestinal, Lymph gland bleeding.	50 mm Hg (6)
26	0	24	300	220	-80	Hemorrhagic diarrhea.	50 mm Hg (30)
28	0	20	335	230	-105	Intestinal bleeding.(-)	—————
29	0	20	330	220	-110	Adrenal gland, intestinal bleeding.	12th day of experiment 250 mm Hg (6) 200 mm Hg (6)
30	0	23	330	270	-60	Intestine, adrenal gland bleeding.	12th day of experiment 250 mm Hg (2)

() number of petechia

TABLE VIII THE INFLUENCE OF "HI" ACID "CHO" SULFATE AND VITAMIN C ON THE CAPILLARY FRAGILITY IN EXPERIMENTAL SCORBUTIC ANIMALS.

No	Body weight	Material administered	Before	3 Hours	6 Hours	12 Hours	18 Hours	24 Hours	30 Hours	48 Hours
141	350 gm.	"hi" acid 5 mg.	50(1) 100(7)	50(0) 100(2)			150(0) 150(0)	50(3) 150(2)		50(1) 100(2)
142	320	"hi" acid 5 mg.	50(2) 100(6)	100(2) 150(2)			150(0) 200(4)	100(2) 150(2)		50(2) 100(7)
143	320	"hi" acid 10 mg.	50(1) 50(7)		150(2) 100(2)				75(1) 75(1)	50(1) 50(3)
145	300	"cho" Sulfate 10 mg.	50(3) 50(3)	50(1)				10(1) 50(2)		
147	220	Vitamin C 100 mg.	50(1)	100(1)		150(1) Vit. C 100mg. administration				250(1)

50() = 50 mmHg

() number of petechiae.

The changes in erythrocytes and thrombocytes of guinea pigs receiving subcutaneous or intravenous injections of anti-thrombocyte serum are shown in Table IX. Hemorrhages were seen in all tissues (Table X). Petehhiae in the lungs, heart, mesenteries, kidneys and subcutaneous tissue were more extensive in Group A than Group B. In group C, only 2 hours after the injection, the sites of injection had already become gelatinous and bloody, and slight bleeding was seen in the lungs, large intestines, kidneys, etc.

Capillary permeability: The capillary permeability was almost the same as in normal animals 30 minutes after the subcutaneous injection of 1 ml of anti-serum, and was slightly increased after 60 minutes, the plasma concentration of T-1824 was about 54 % at 90 minutes, as compared with 62.82 ± 3.67 % in normal guinea pigs. (Table XI) That is, a gradual increase in capillary permeability started one hour after the injection. Guinea pigs received subcutaneous injections of anti-thrombocyte serum one hour after subcutaneous

TABLE IX COUNTS OF ERYTHROCYTES AND THROMBOCYTES AFTER INJECTION OF ANTITHROMBOCYTE SERUM.

			Before	15 minutes	30 minutes	2 hours	4 hrs	24 hours
2	Subcutaneous injection	Erythrocytes	5.50 million	5.30	-	4.60	-	4.00
		Thrombocytes	274,000	143,000	133,000	23,000	-	9,800
3		Erythrocytes	5.40 million	4.85	-	4.30	-	3.85
		Thrombocytes	323,000	115,000	863,000	178,000	-	12,000
4	Intravenous injection	Erythrocytes	5.32 million	4.38	4.30	2.30	-	3.30
		Thrombocytes	345,000	9600	1200	9800	-	98,000
5		Erythrocytes	4.87 million	4.70	4.74	4.00	-	3.95
		Thrombocytes	332,000	13,000	8900	10,100	-	54,000

TABLE X HEMORRHAGES IN GUINEA PIGS AFTER SUBCUTANEOUS INJECTIONS OF ANTITHROMBOCYTE SERUM.

GROUP A DAILY SUBCUTANEOUS INJECTION FOR 2 DAYS, 24 HOURS AFTER THE LAST INJECTION.

GROUP B 24 HOURS AFTER SINGLE SUBSTANEOUS INJECTION OF ANTISERUM.

GROUP C 2 HOURS AFTER SINGLE SUBCUTANEOUS INJECTION OF ANTISERUM.

	No of guinea pig	Quantity injected	Hours of survival	Subcutaneous tissue	Heart	Lung	Pleura	Diaphragm	Liver	Kidney	Adrenal gland	Large intestine	Small intestine	Mesentery	Peritoneum	Uterus	Urinary bladder	Stomach
Subcutaneous injection A	8	1.0 1.0	48 hrs.	+++	+	++	-	-	+	+	+	-	-	-	-	-	-	+
	5	1.0 1.0	48	+++	+	+	+	-	-	+	-	+	+	-	+	+	-	+++
	9	1.0 1.0	48	+++	+	+++	+	-	+	+	+	+	+	-	-	-	-	+
	11	1.0 1.0	24	+	blood vessel dilatation	+	-	-	-	-	-	-	blood vessel dilatation	-	-	-	-	blood vessel dilatation
Injection B	12	1.0 1.0	24	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-
	13	1.0	24	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+
	15	1.0	24	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+
Injection C	14	1.0	24	+	-	++	-	-	-	-	-	+	+	-	-	-	-	+
	17	1.0	2	+	-	+	-	-	-	-	-	+	-	-	-	-	-	+
	18	1.0	2	+	-	+	-	-	-	-	-	+	+	-	-	-	-	+

TABLE XI CAPILLARY PERMEABILITY OF GUINEA PIGS AFTER SUBCUTANEOUS INJECTIONS OF ANTITHROMBOCYTE SERUM ALONE OR PRETREATED WITH "HI" ACID OR "CHO" SULFATE.

	Normal	Subcutaneous injection of antithrombocytes serum alone	Pretreated with "hi" acid. 5 mg daily (subcutan.)	Pretreated with "cho" sulfate. 10 mg daily (subcutan.)
30 minutes	84.83 $\pm$ 6.46	82.45 $\pm$ 3.76	81.08 $\pm$ 5.87	80.87 $\pm$ 4.86
60 minutes	67.47 $\pm$ 2.63	62.62 $\pm$ 6.31	69.89 $\pm$ 6.64	61.11 $\pm$ 7.46
90 minutes	62.82 $\pm$ 3.67	54.1 $\pm$ 4.31	54.05 $\pm$ 4.24	55.55 $\pm$ 6.43

TABLE XII THE PRECAPILLARY FRAGILITY OF GUINEA PIGS RECEIVED SUBCUTANEOUS INJECTIONS OF ANTITHROMBOCYTE SERUM ALONE OR PRETREATED WITH 5 MG OF "HI" ACID OR 10 MG OF "CHO" SULFATE DAILY 5 TIMES.

Number	Sex	Body weight	Pre-treat-ment	Before	30 minutes	60 "	90 "	120 "	150 "	180 "	210 "	240 "	Bleeding area
102	o	250	-	300(0)		200(2)		200(4)		200(1)		200(1)	(+)
104	o	240	-	300(0)	300(1)	200(5)		200(3)		200(1)		200(3)	(+)
105	o	270	-	300(0)		300(1)		200(2)		200(2)			(+)
109	o	230	"hi" 5mg x 5	300(0)	300(0)	300(0)	300(0)	300(8)					(-)
110	o	240	"	300(0)	300(0)	300(0)	300(5)	200(3)	200(3)				(-)
131	o	220	"	300(0)	300(0)	300(1)	250(0)	250(3)					(+)
111	o	270	"cho" sulfate 10mg x 5	300(0)	300(0)	250(3)	200(1)	200(7)	300(3)				(+)
122	o	270	"	300(0)	-	250(1)		200(1)		200(5)	150(0)		(+)
130	o	270	"	300(0)	-	200(1)		200(3)		250(1)			(+)

300(0) = 300 mm Hg (Number of petechiae)

TABLE XIII COUNTS OF ERYTHROCYTES AND THROMBOCYTES AFTER INJECTION OF ANTIENDOTHELIAL SERUM.

			Before	$\frac{1}{2}$ hours	$\frac{1}{2}$ hours	2 hours	4 hours	24 hours
2	subcutaneous injection	Erythrocyten	5.40 million	5.25	5.60	5.45	-	5.38
		Thrombocyten	274,000	235,000	283,000	245,000	-	234,000
3		Erythrocyten	5.25 million	5.32	5.14	-	-	5.30
		Thrombocyten	296,000	-	266,000	-	-	287,000
4	intravenous injection	Erythrocyten	5.35 million	5.50	4.90	-	5.18	5.01
		Thrombocyten	276,000	214,000	224,000	223,000	264,000	254,000
6		Erythrocyten	4.65 million	4.35	4.45	4.25	4.85	4.90
		Thrombocyten	246,000	224,000	215,000	204,600	212,000	234,000

injection of 5 mg of "hi" acid daily for 5 days. The capillary permeability in these treated animals was near the standard range 69.89 % after 60 minutes, but showed the same increase (54%)

as in the untreated group which was injected with anti-serum after 90 minutes. In the cases treated with "cho" sulfate daily for 5 days, there was the same degree of capillaay permeability as in

the untreated cases.

Capillary fragility: In observing capillary fragility and bleeding in the depilated areas, as shown at Table XII, in the group which was treated with daily subcutaneous injections of "hi" acid for 5 days there seemed to be an inhibition in the increase of capillary fragility as compared with the untreated group. That is, the capillary fragility in the untreated group was observed at 200 mmHg on the chest after 60 minutes, while in the group treated with "hi" acid was it over 300 mmHg. After 120 minutes the capillary fragility was at 250 mmHg in the latter group, which showed more capillary resistance than the untreated cases whose value remained 200 mmHg. In the "cho" sulfate treated cases, the capillary fragility increased 60 minutes after the injection. And in the "hi" acid treated cases, the bleeding in the area of depilation was decreased.

No histochemical changes was observed by Toluidin blue and P.A.S. staining method in the blood vessel wall compared with those in the control animals.

- 4) The influence of acid polysaccharides on the capillary permeability and fragility in cases of experimental vascular purpura.

When guinea pigs received subcutaneous injections of 1 ml of anti-endothelial serum (Table XIII), a slight decrease in the number of thrombocytes developed.

At autopsy a gelatinous substance and petechiae were recognized at the site of injection,

and petechiae were also in subcutaneous tissue, lung, heart and large intestines (Table XIV). The petechiae showed no difference between group B (24 hours after one injection) and group C (2 hours after one injection).

Permeability: As shown in Table XV, the concentration of T-1824 in the serum of the experimental vascular purpura, was 95.08 % after 30 minutes, while 83.8 % in the serum of normal guinea pigs, the former showing a decrease in permeability. At 60 minutes the permeability of the experimental group showed as light increase i.e., 65.43 % as compared with 67.47 % for the normal group, and at 90 minutes the former showed 56.42 % of increase in permeability as compared with 62.82 % in the latter. In comparing the untreated group and the groups receiving subcutaneous injections of "hi" acid daily for 5 days and 1.0 ml of anti-endothelial serum subcutaneously one hour after the last injection, 30 minutes later the capillary permeability in the latter was 91.08 %, i.e. a slight increase in comparison with 95 % in the former. But the permeability of both groups was decreased in comparison with the value of the normal group (84.83 %). The permeabilities of untreated, treated and normal group, 60 minutes after injection, were respectively 65.4 %, 67.29 % and 67.47 %. Capillary permeability in the untreated group (57.42 %) and the treated group (56.68 %) was almost the same after 90 minutes, but both groups showed increased capillary permeability in comparison with the normal group (62.82 %). The

TABLE XIV HEMORRHAGES IN GUINEA PIGS RECEIVED SUBCUTANEOUS OF ANTIENDOTHELIAL SERUM.

GROUP A DAILY SUBCUTANEOUS INJECTION FOR 2 DAYS.

GROUP B 24 HOURS AFTER SUBCUTANEOUS INJECTION OF ANTISERUM.

GROUP C 2 HOURS AFTER SUBCUTANEOUS INJECTION OF ANTISERUM.

		Number of guinea pig	Quantity injected	hours of survival	Subcutaneous tissue	Heart	Lung	Small intestine	Stomach	Kidney
Subcutaneous injection	A	8	1.0 1.0	48 hrs.	++	+	±	-	+	-
		9	1.0 1.0	" "	++	±	+	+	±	+
		10	1.0 1.0	" "	+	-	+	+	±	-
		11	1.0 1.0	" "	++	+	±	-	-	-
		12	1.0 1.0	" "	+	÷	+	+	÷	-
	B	13	1.0	24 hrs.	+	±	-	-	-	-
		14	1.0	" "	+	-	+	-	-	-
		15	1.0	" "	+	-	-	-	-	-
	C	17	1.0	2 hrs.	+	±	-	-	-	-
		18	1.0	" "	+	-	-	-	-	-

TABLE XV THE INFLUENCE OF "HI" ACID OR "CHO" SULFATE ON PRECAPILLARY PERMEABILITY IN EXPERIMENTAL VASCULAR PURPURA.

	Normal	Anti-endothelial serum	"hi" acid 5mg. daily subcutaneous injection for 5 days	"hi" acid 5mg. intravenous injection single	"cho" sulfate 10mg. daily subcutaneous injection for 5 days
30 minutes	84.83 ± 6.46	95.08 ± 7.10	91.08 ± 7.80	96.7	93.08 ± 6.58
60 minutes	67.47 ± 2.63	65.43 ± 3.36	67.29 ± 6.93	67.46	66.45 ± 5.36
90 minutes	62.82 ± 3.67	57.42 ± 4.58	56.68 ± 5.38	56.45	57.32 ± 6.68
120 minutes		51.45 ± 5.39	51.10 ± 5.26	49.92	50.48 ± 6.5

TABLE XVI CAPILLARY FRAGILITY OF GUINEA PIGS WHICH RECEIVED SUBCUTANEOUS INJECTIONS OF ANTI-ENDORHEL SERUM ALONE OR PRETREATED WITH "HI" ACID OR "CHO" SULFATE.

Number	Sex	Body weight	Pretreatment	Before	30'	60'	90'	120'	150'	180'	210'	240'	Bleeding of depilated area
106	o	240	--	300(0)	-	200(1)	-	200(1)	-	200(2)	-	-	
107	o	250	--	300(0)	300(1)	200(1) 100(1)	-	150(5) 100(1)	-	-	-	-	
108	o	250	--	300(0)	-	200(1)	200(5)	150(1)	200(1)	-	-	-	
116	o	260	--	300(0)	300(1)	250(8) 200(0)	250(1)	250(1)	300(0)	-	-	300(0)	
114	o	250	"hi" acid 5 mg 5 days	300(0)	300(0)	300(0)	300(6) 200(1)	300(1)	300(0)	300(0)	-	-	
115	o	240	"	300(0)	300(0)	300(0)	300(1)	300(0)	300(0)	300(0)	-	-	
118	o	250	"	300(0)	300(0)	300(0)	300(0)	300(0)	-	-	-	-	
113	o	320	"hi" acid 5 mg intravenous injection	300(0)	300(4) 200(2)	200(2) 100(6)	100(6) 50(0)	200(1) 100(0)	200(1)	200(1)	200(1)	200(1)	
112	o	230	"hi" acid 5 mg 5 days	300(0)	300(0)	300(0)	300(3) 200(0)	300(2)	-	300(0)	-	300(0)	
128	o	260	"cho" sulfate 10 mg 5 days	300(0)		250(1)	-	200(3)	-	-	-	-	
119	o	250	"	300(0)		250(4) 200(0)	200(1)	200(5)	-	-	-	-	

300() = 300 mmHg (number of petechiae.)

"cho" sulfate treated group showed almost same results as the "hi" acid treated one. In short, no significant difference in capillary permeability could be recognized between the treated group ("hi" acid and "cho" sulfate) and the untreated group.

Capillary fragility: The untreated group showed increased capillary fragility from the time of 60 minutes after injection of anti-endothelial serum (in Table XVI). The group which received daily injections of "hi" acid for 5 days showed no increase in capillary fragility at 60 minutes after injection of anti-endothelial serum, (in guinea pigs which received injections of anti-endothelial serum 1 hour after subcutaneous injection of "hi"

acid.), and capillary fragility was more than 300 mmHg. After 120 minutes most of them showed 300 mmHg or more, and they clearly showed decreased fragility in comparison with the untreated group. One case which received an injection of anti-endothelial serum immediately after one intravenous injection of 5 mg of "hi" acid showed increase in capillary fragility similarly to the untreated group. Each of the group injected with "cho" sulfate showed an increased capillary fragility of 200-250 mmHg after 60 minutes and 200 mmHg after 120 minutes similarly to the untreated group. The group which received subcutaneous injections of "hi" acid tended to have a slight decrease in bleeding in the depilated parts.

Histochemically the acid polysaccharides of blood vessel walls showed no difference from the normal group.

DISCUSSION

Metcoff reported that when white rats received intravenous injections of T-1824 dyes, 3 to 5 minutes were for complete mixing of the dyes in the blood stream³⁷. Therefore the author removed blood specimen from jugular vein of guinea pigs 5 minutes after the injection, and considered the plasma dye concentration at this time to be 100 %. In regard to the effect of "cho" sulfate on capillary permeability, Nomura reported that capillary permeability was decreased by "cho" sulfate³⁹, while Oshima recognized the vasodilating action of "cho" sulfate⁴¹. When the author injected daily 10 mg of "cho" sulfate subcutaneously or intravenously, in neither tests could the author recognize any increase or decrease in capillary permeability as compared to a normal guinea pig. "hi" acid gave the same results. There are many reports about the influence of hyaluronidase on capillary permeability. Elster recognized that intravenous injection of hyaluronidase increased capillary permeability in white rats, and this difference was most pronounced when the plasma concentration of T-1824 at 30 minutes after injection was compared with the control group, after that the difference between the two groups diminished, and it was not significant after 8 to 12 hours^{17,18}. Heinlein and Braun reported that intravenous injection of hyaluronidase increased capillary permeability^{4,22}. On the other hand Hubner said that hyaluronidase did not move into capillaries after subcutaneous injection²⁴. Benditt said that the more hyaluronidase became pure, the less it increased capillary permeability⁵. Zweifach and chamber said that they observed in none of their cases an increase in permeability after intravenous injection of hyaluronidase^{10,58}. It is difficult to make comparison on the quantitative relations of hyaluronidase because there is no standardized International Unit for hyaluronidase

yet available. In the author's test results, when guinea pigs received intravenous injections of 20,000 V.U.M. hyaluronidase (Motida & Co. 33,500 V.U.M. per mg of nitrogen), as shown in Table 3, capillary permeability in guinea pigs became slightly increased in comparison with the control group at 30 minutes after the injection, but after that it returned within the normal range. The author thinks this result is due to the following points:

1) there is enzymatic activity in the blood within 30 minutes after the intravenous injection of hyaluronidase 20,000 V.U.M., but after that time this is disturbed by an inhibitor in the blood, or 2) there is a secondary sudden increase in permeability due to vasodilatation, or 3) this hyaluronidase includes a small amount of a permeability increasing factor.

There have been many reports on experimental scurvy^{31,27,56,33,9,34,26,19}. Elster reported that he injected 1 % T-1824 solution intravenously in experimental scorbutic guinea pigs and removed blood from them after 5 minutes, 30 minutes, 60 minutes, 120 minutes and 240 minutes, and he found no increase in capillary permeability using Philip's method¹⁹. Imagawa²⁶ injected egg albumin into the veins of experimental scorbutic animals and observed the quantity moving into their ascitic fluid. He found increased capillary permeability in experimental scurvy. In the author's test, experimental scorbutic animals showed an obvious increase in capillary permeability in comparison with normal animals and a marked increase in capillary fragility. In the group receiving Vitamin C injection daily there was a decrease in capillary permeability, but one hour after the intravenous injection of Vitamin C 250 mg on the 22nd experimental day there was no improvement in permeability. Imagawa reported the same results²⁶. Capillary fragility was obviously improved 3 hours after the subcutaneous injection of Vitamin C 100 mg. Pasqualine stated that shock by bleeding in guinea pigs could be prevented

by the injection of Vitamin C 200 mg⁴³⁾. Wolbach said that the formation of capillary cement substance regulates Vitamin C⁵⁶⁾, but Chamber said that the formation of intercellular cement substance in the tissue culture does not always need Vitamin C¹³⁾. Daldorf¹⁵⁾ said that the capillary fragility of patients with clinical scurvy improved 45 minutes after intravenous injection of Vitamin C 100 mg. Moreover, Hines said that the injection of large amounts of Vitamin C cured the increased capillary fragility for 10 to 30 minutes²³⁾. Also in the author's test, the increase in capillary fragility showed rapid improvement after the injection of Vitamin C. The "hi" acid treated group showed a tendency to improve the increased capillary fragility and permeability. But in the "cho" sulfate treated group there was no improvement. "hi" acid and Vitamin C seemed to exert a good influence on the increased capillary fragility and permeability in experimental scurvy. In histo-chemical studies of blood vessel walls, Bunting⁶⁾ said that the M. substance in the wound of incompletes scorbutic animals was increased as compared with that of normal animals, and Penney said that M. substance in the intercellular space in granulation tissue decreased or disappeared in scorbutic animals⁴⁴⁾. According to Sadhu⁴⁸⁾, "cho" sulfate in the skin and bone marrow decreased in scurvy. Kaneko referred to the elastic fibrils of the blood vessel wall²⁷⁾, and Imagawa also noted roughness, fibrinoid swelling, vacuolation, and cell infiltration in the blood vessel wall with hematoxylin-eosin stains²⁵⁾. There was no recognizable difference between normal and scorbutic animals with P.A.S. and Toluidin-blue staining methods.

There has been many reports on experimental thrombocytopenic purpura³²⁾⁵¹⁾⁷⁾⁸⁾⁵²⁾⁵³⁾²⁹⁾⁵⁵⁾²⁸⁾⁴⁹⁾¹⁶⁾⁴⁷⁾. Elliott¹⁶⁾ said that the increase in capillary fragility reached its peak 24 hours after the injection of anti-thrombocyte serum, and for 7 days thereafter the same degree of fragility was

maintained. Though the observation period was only 4 hours in the author's experiments, they showed an increase in capillary fragility 60 or 120 minutes after injection and maintained the same degree for 4 hours. As stated in the autopsy findings, at sites of injection there was a gelatinous substance and petechiae 2 hours after the injection, and bleeding in the internal organs. Kawano²⁸⁾ reported that diapedesis was present throughout the body 2 hours after the abdominal injection of anti-thrombocyte serum. Though there are some differences in resorption time between subcutaneous injection and intraperitoneal injection, it is definite that the bleeding begins early. In the "hi" acid treated groups, the increase in capillary permeability and fragility seemed to be inhibited to some degree as compared with the untreated groups at 60 minutes after antiserum injection. Randall⁴⁷⁾ gave rats subcutaneous injections of 1 ml anti-thrombocyte serum, and at the same time daily oral or intraperitoneal injection of Vitamin P. for 2 or 3 days. The capillary fragility was less than in the untreated group when measured 6 hours after the last administration of Vitamin P. In addition, he reported that capillary fragility of rats was lowered by Rutin, adrenochrome, etc. Hiramatsu said that bleeding could be slightly decreased by preliminary treatment with Vitamin P for 5 days daily²¹⁾. In short, "hi" acid and Vitamin P seems to have good effect on capillary resistance. Kawano said that in thrombopenic purpura the nuclei of smooth muscle cells in blood vessel walls were slightly swollen, and muscle cells showed vacuolar degeneration²⁸⁾. In the staining of acid polysaccharides in the blood vessel wall, the author did not recognize any differences from the normal cases.

It should be noted that permeability of experimental vascular purpura was decreased for the first 30 minutes, and then increased with the lapse of time. Katsura said that there was swelling of endothelial cells in experimental vascular purpura, and also desquamation of endothelial cells³⁰⁾.

Taking into consideration the above findings, it seems probable that the decrease in permeability is first caused by a reduction of intercellular space because of swelling of endothelial cells accompanying antigen-antibody reaction, and then increased permeability is caused by the cracking of intercellular space due to poor formation of intercellular cement substance accompanying the reduction of endothelial cell function and the desquamation of endothelial cells. Hiramatsu says that Vitamin P suppresses the appearance of petechiae in experimental vascular purpura caused by injection of antiendothelial serum²⁾. Sawada recognized a little decrease of capillary resistance following antiendothelial serum injection in rabbits⁴⁹⁾. The author found that "hi" acid decreased bleeding in the depilated areas and increase of capillary fragility. Capillary fragility and capillary permeability showed no difference between the group which received injections of "cho" sulfate and the untreated group. Among the histological changes revealed in blood vessels after injection of antiendothelial serum, Katsura recognized swelling and desquamation of endothelial cells in blood vessel walls³⁰⁾. Studying polysaccharides in blood vessel walls by histo-chemical methods, the author could not recognize any difference between the normal group and the experimental vascular purpura group.

Amano and Kawano said that diapedesis in purpura caused by antithrombocyte serum was caused by bleeding from venules and not from capillaries²⁸⁾. Using a capillary microscope, Hamble found that bleeding occurred at the arteriolo-capillary junction at the time of measuring the capillary fragility²⁰⁾. In thrombotic thrombocytopenic purpura, Orbison found that bleeding occurred on rupture of aneurysma at the arteriolo-capillary junctions, and not from veins and venules⁴²⁾. Therefore even in regard to the site of bleeding there is no agreement. The details of the mechanism of bleeding are even less clear. According to Chamber¹⁰⁾, capillaries are formed of three important

structures. The first, the endothelium does not control capillary directly. Endothelial cells perhaps secrete a cement substance between the cells, and perhaps changes in this cement substance may cause changes in permeability. The second important structure is the endo-sheath of the capillary. It is covered and lined with endothelial cells, and formed by an adaptation of blood protein. Perhaps changes in the endo-sheath of capillaries cause an increased capillary permeability. The third structure is the connective tissue of the pericapillary-sheath. This pericapillary-sheath may contain "hi" acid, and this perhaps has some influence on capillary permeability. Reppert states that the rapid recovery of increased capillary fragility following the administration of Vitamin C is due to the more influence on "hi" acid in the pericapillary-sheath than to the cement substance between cells⁴⁶⁾. Cella considers "hi" acid, Vitamin C, and Vitamin P to be presubstances of capillary cement substance. In every experiments the author recognized that the increase of capillary fragility and of capillary permeability decreased on administration of "hi" acid. Moreover capillary permeability increased after intravenous injection of hyaluronidase. Hines said that the increase of capillary fragility was corrected quickly on administration of large quantities of Vitamin C²³⁾, and Hiramatsu said that in cases of experimental thrombocytopenic purpura and experimental vascular purpura the bleeding was decreased more by Vitamin P than by Vitamin C²³⁾. Capillary permeability is controlled by many conditions. Abell says that new capillaries are more permeable to T-1824 than old ones¹⁾. The relationship between capillary fragility and permeability was studied. 90 minutes after the injection of antithrombocyte serum the fragility was 200 mmHg, and in the cases receiving injections of anti-endothelial serum the fragility was also 200 mmHg. The capillary fragility of the experimental scorbutic animal group was 30-50 mmHg. With capillary permeability, however 90 minutes after the

intravenous injection of T-1824 its density was 54 % in thrombocytopenic purpura, 57 % in vascular purpura, and 52 % in scurvy. The capillary fragility of experimental scorbutic animals showed a considerable increase in comparison with the other experimental purpura animals, but the permeability scarcely increased at all in comparison with the other purpuras. 30 minutes after the injection of anti-endothelial serum capillary permeability was clearly inhibited, but capillary fragility showed a tendency to increase slightly. As described above, capillary fragility and permeability are not always parallel. Zweifach reported that he could not find any increase in capillary permeability before the rupture of capillaries in the mesenteries⁵⁸⁾. In the observation of rabbit's ear blood vessels, Clark¹²⁾ reported similar findings. Landis³⁵⁾³⁶⁾, too, differentiated between capillary fragility and permeability, and considered that they were not related. The author's experimental results were the same as these author's. There was observed no significant difference from normal animals when the polysaccharides in the blood vessel wall of animals were examined histo-chemically with various experimental hemorrhagic disease. Capillaries and precapillary blood vessels did not show Metachromasia with the Toluidin blue staining method, but were stained by P.A.S. staining method. M. substance in large and small arteries was the same as in normal animals. This result indicates that M. substance in blood vessels, that is, "cho" sulfate is not related to hemorrhagic diathesis, or that the method of examination is not accurate enough.

SUMMARY

- 1) The normal rats groups receiving "hi" acid and "cho" sulfate did not show either increase or decrease in capillary permeability.
- 2) Capillary permeability increased 30 minutes after the intravenous injection of hyaluronidase 20,000 V.U.M. (33,500 R.U.M. per mg of Nitrogen).
- 3) Scorbutic animals showed a greater

increase in capillary permeability than normal animals did. Experimental scorbutic animals showed only a little increase in capillary fragility after 12 days, but after 20 days they show a very definite increase. Treatment with Vitamin C and "hi" acid improved capillary permeability and fragility of experimental scorbutic animals, but no improvement was seen in the "cho" sulfate-treated groups.

4) "hi" acid seemed to inhibit the increase of capillary fragility in experimental purpuric guinea pigs. It also caused a slight suppression in the increase of capillary permeability. In the cases treated with "cho" sulfate, neither the fragility nor the permeability is different from the untreated cases.

5) After subcutaneous injection of 1 ml of anti-endothelial serum, capillary permeability decreased for 30 minutes and then increased. Increased capillary fragility in experimental vascular purpura was decreased by injection of "hi" acid.

6) Capillary fragility and permeability do not always parallel each other. In experimental vascular purpura, when the capillary fragility began to increase, the capillary permeability decreased.

7) The acid polysaccharides in blood vessel walls in animals with experimental hemorrhagic diathesis were observed to be approximately the same as in normal animals by means of Lieson's method.

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