16. Saburo Hara and Jun Sato: On the Nature of Anticoagulant Action of Rare Earth Metals.

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Anticoagulation agents are now being clinically used and Heparin and Dicumarol are the most widely used anticoagulants in the therapy of thrombosis and embolism. Recently, Thromexan** and Paritol*** have been marketed.

Pharmacological studies on the rare earth metals have been carried on in this Laboratory for the past thirty years. Hara had earlier noticed the anticoagulant action as the specificity of rare earth metals[1] and carried out fundamental experiments on the nature and therapeutic significance of such specific action[2~5] which are reported in the presented paper.

Experimental Methods

Material: As rare earth metals, hydrochlorides of the cerium series (lanthanum, cerium, praseodymium, and neodymium) and the yttrium series (yttrium) of the most pure product, purified by Deutsche Gasglühlich Auer Gesellschaft before the recent world war, were used.

Test Animal: Mature rabbits.

Following tests were carried out *in vitro* (I) and *in vivo* (II):

1) Measurement of time required for blood clotting.
2) Change of coagulation factors.
3) Relationship between coagulants.
4) Relationship between anticoagulants.

Besides the above, histological change of liver tissues was examined because of its close relationship to blood coagulation.

Results

I. Anticoagulant Action *in vitro*

1) Measurement of Clotting Time—Measured by the test tube method in a thermostat of 37°. All the metals tested showed inhibition of rabbit blood coagulation at 0.05% and complete inhibition at above 0.2% (cf. Table I).

TABLE I. Anticoagulant Action of Rabbit Blood *in vitro*

Compd.	Concn. (%)						
	0.01	0.05	0.075	0.1	0.15	0.2	0.3
$LaCl_3$	N	N	+	++	+++	+++	++++
$CeCl_3$	N	N	+	++	+++	+++	++++
$PrCl_3$	N	N	+	++	+++	+++	++++
$NdCl_3$	N	N	+	++	+++	+++	++++
YCl_3	N	+	+	++	+++	+++	++++

N: coagulation within 5 mins., +: within 10 mins., ++: within 20 mins., +++: within 30 mins., ++++: complete inhibition.

Relationship between the Anticoagulant Activity of Rare Earth Metals and Coagulation Agents: Such a relationship was tested with Throgestin (Torii), biological coagulant preparation containing 22.5 mg. of thrombin in 2 cc., and vitamin K. In fresh blood from a rabbit, the inhibition of blood coagulation by 0.75 mg./cc. of the metals was hindered by 0.75 mg./cc. of Throgestin and by 1.0 mg./cc. of vitamin K (cf. Table II).

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** Trade name for ethyl biscoumacetate.
*** Trade name for sodium polyanhydromannuronic acid.

1) S. Hara: Arch. exptl. Path. Pharmakol., **100**, 217(1923).
2) I. Mori: J. Exptl. Pharmacol. (Japan), **20**, 117(1943).
3) K. Kono: Folia Pharmacol. Japon., **38**, 2(1942).
4) J. Sato: *Ibid.*, **49**, 2(1953).
5) T. Agawa: J. Tokyo Med. Coll., **12**, 1(1954).

Table II. Action of Coagulation Agents on Inhibition of Blood Coagulation by 0.2 mg./cc. of $CeCl_3$

	Coagul. agent (mg./cc.)	Time of clotting (min.)	Control (min.)		Coagul. agent (mg./cc.)	Time of clotting (min.)	Control (min.)
Throgestin	0.025	10.00	11.00	Vitamin K	0.025	15.00	10.00
	0.04	7.00			0.05	9.50	
	0.06	7.00			0.1	4.00	
	0.1	5.00			0.2	3.00	

Relationship between Anticoagulation Activity of Rare Earth Metals and That of Other Anticoagulants: Such relationship was examined with Heparin (Roche) by measuring the time required for clotting of the blood after addition of one-half the minimum amount of Heparin and each of the rare earth metals tested necessary to cause inhibition of coagulation but no prolongation of the clotting time was observed.

II. Anticoagulant Action *in vivo*

Inhibition of blood coagulation appeared on intravenous injection of over 15 mg./kg. of the metals in mature rabbit (cf. Table III).

Table III. Inhibition of Blood Coagulation *in vivo* (Mature rabbits; intravenous injection)*

Compd.	Amt. given (mg./kg.)	Before inj.	After injection				
			5 mins.	30 mins.	1 hr.	3 hrs.	24 hrs.
$LaCl_3$	10	N	N	N	N	N	N
	20	N	+	++	++	N	N
	40	N	++	+++	+++	++	N
$CeCl_3$	10	N	N	N	N	N	N
	20	N	+	++	++	N	N
	40	N	++	+++	+++	++	N
$PrCl_3$	10	N	N	N	N	N	N
	20	N	+	++	++	N.	N
	40	N	++	+++	+++	++	N
$NdCl_3$	10	N	N	N	N	N	N
	20	N	+	++	++	N	N
	40	N	++	+++	+++	++	N
YCl_3	1	N	N	N	N	N	N
	5	N	+	+	N	N	N
	10	N	+	+	+	N	N
	20	N	++	+	+	N	N
	50	N	+++	++	+	+	N

* All notations are the same as in Table I.

However, such action did not appear by intramuscular injection or oral administration, even in a large dose.

Behavior of Various Coagulation Factors on the Anticoagulation Activity of Rare Earth Metals: The amount of prothrombin, thrombin, fibrinogen, antithrombin, serum calcium, and thrombocytes were measured 5 mins., 30 mins., 1 hr., and 3 hrs. after intravenous injection of 20 mg./kg. of each of the metals in normal, mature rabbits and the results were as follows:

Prothrombin Time: Measurement was carried out on total plasma by the Quick-First Step method. The prothrombin time prolonged 5 mins. after injection, became marked after 30 mins., and returned approximately to normal in 3 hrs. (Fig. 1, (a)).

Amount of Thrombin: Measured by the Wohlgemuth method. Decreased 5 mins. after injection, became marked after 30 mins., and returned to normal in 3 hrs. (Fig. 1, (b)).

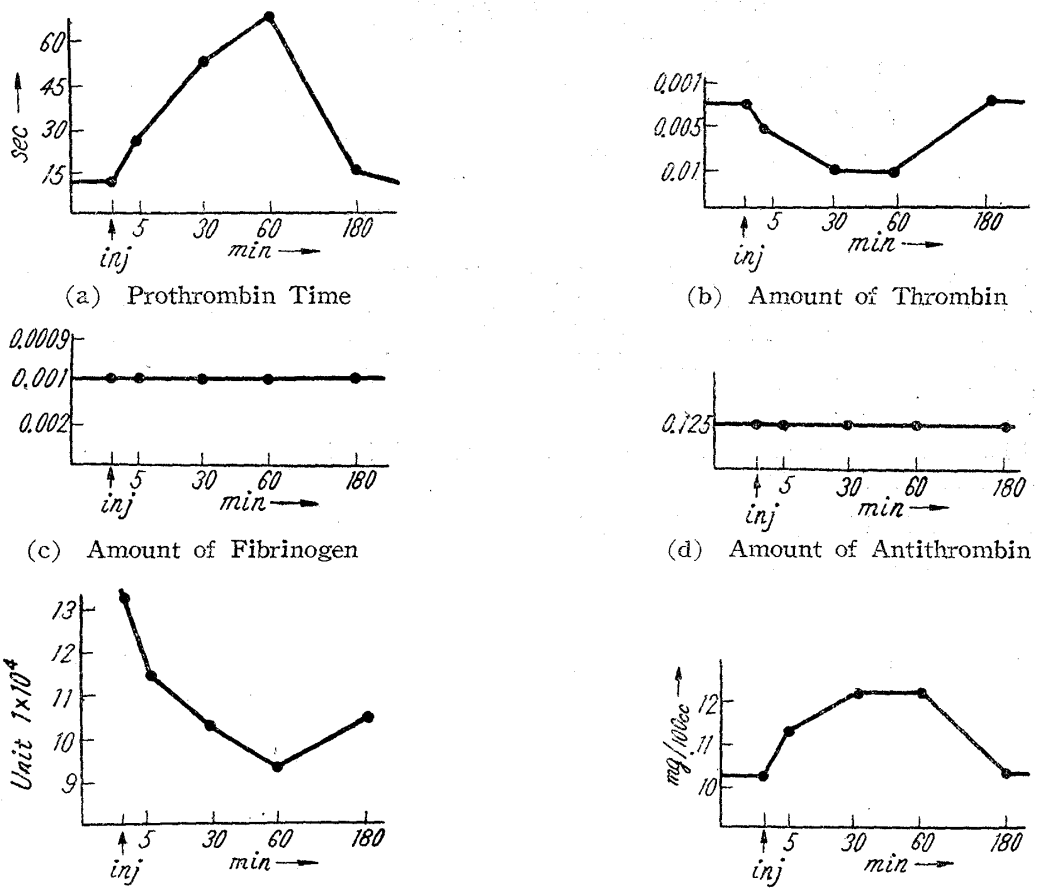
Amount of Fibrinogen: Measured by the Wohlgemuth method. There was no observable change in the amount of fibrinogen after the injection (Fig. 1, (c)).

Amount of Antithrombin: Measured by the Wilson method, a modification of the Howell-Hess method. There were no observable change in the amount of antithrombin after the injection (Fig. 1, d)).

Amount of Serum Calcium: Measured by the Clark-Collip method. There was a slight, transitory increase in the amount of serum calcium after the injection (Fig. 1, (e)).

Number of Thrombocytes: Measured by the Fonio method. A decrease of thrombocytes was observed after the injection (Fig. 1, (f)).

Relationship between the Anticoagulation Activity of Rare Earth Metals and Anticoagulants: Such a relationship was examined between the metals and Thrombrin (Motida), a biological coagulation



(a) Prothrombin Time (b) Amount of Thrombin

(c) Amount of Fibrinogen (d) Amount of Antithrombin

(e) Amount of Serum Calcium (f) Number of Thrombocytes

Fig. 1. Change of Various Coagulation Factors after Injection of 20 mg./kg. of $CeCl_3$ into Aural Vein of a Rabbit

agent (containing 20 mg. of thrombin), and vitamin K. After intravenous injection of 20 mg./kg. of each metal into a rabbit, when the clotting time was prolonged to 15 mins., 20 mins. after the injection, 5 cc. Thrombrin was given by intravenous injection by which the anticoagulation activity was inhibited 10 mins. after the injection. Vitamin K, in 20 mg./kg. dose, inhibited the anticoagulation activity of the rare earth metals tested (Table IV).

Table IV. Action of Various Coagulation Agents on the Inhibition of Blood Coagulation by 0.75 mg. of $CeCl_3$

	Coagul. agent (mg./cc.)	Time of clotting (min.)	Control (min.)		Coagul. agent (mg./cc.)	Time of clotting (min.)	Control (min.)
Thrombrin	0.32	15.00	15.00	Vitamin K	0.5	15.00	18.00
	0.75	6.00	10.00		1.0	5.00	
	0.75	5.00			1.5	5.00	
	1.5	3.00			2.0	3.00	

Relationship between Anticoagulation Activity of Rare Earth Metals and Other Anticoagulants: Intravenous administration of a mixture of one-half the minimum amount of Heparin (Roche) and the rare earth metals necessary to effect inhibition of blood coagulation in rabbit failed to cause such inhibitive action.

On the other hand, oral administration of Dicumarol followed by intravenous injection of the rare earth metals in one-half the dose necessary to inhibit coagulation resulted in acceleration of anticoagulating action. Consecutive oral administration of 20 mg./kg. of Dicumarol for two days resulted in the shortening of coagulation time to 15 mins. On subsequent intravenous injection of 5 mg./kg. of one of the rare earth metals, complete coagulation occurred 30 mins. after the injection.

Effect of Rare Earth Metals on Liver Tissues: Consecutive intravenous administration of the metals of cerium series in a rabbit for 6~7 days results in the damage of liver functions. Patholohistological observations revealed necrosis and fatty infiltration of the liver tissues, as shown in Fig. 2.

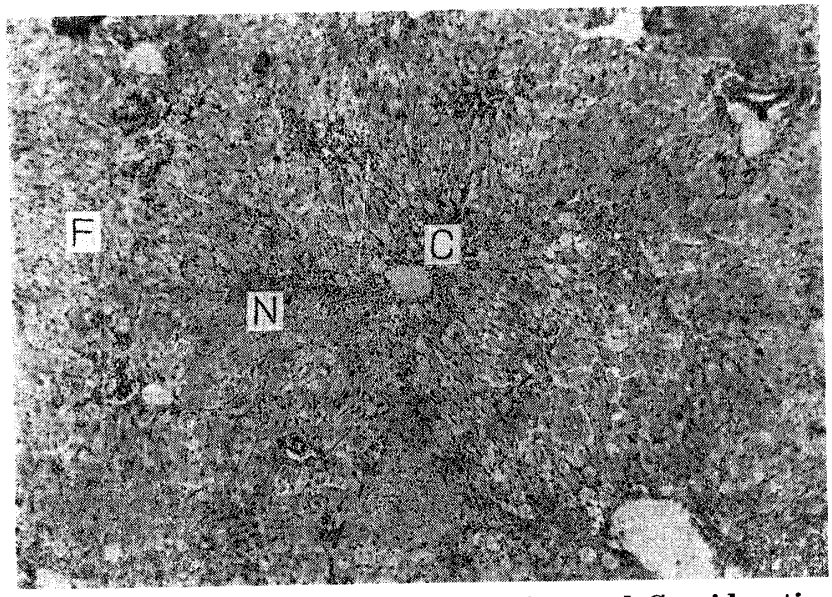


Fig. 2. Liver Tissues of a Rabbit after Intravenous Injection of Cerium

C : Central vein
F : Fatty infiltration
N : Necrosis

Conclusion and Considerations

From the foregoing experimental results, the action mechanism of rare earth metals in inhibiting blood coagulation was considered as follows, based on the hypothesis of Morawitz and Wohlisch.

I. *In vitro* Action Mechanism—The action of rare earth metals in inhibiting blood coagulation *in vitro* is antagonized by vitamin K, which accelerates activation from prothrombin to thrombin, synergetic with Heparin, which has an antithrombin action, and is unrelated to calcium. These facts indicate that the rare earth metals destroy prothrombin in blood and inhibit its activation to thrombin.

II. *In vivo* Action Mechanism—Inhibition of blood coagulation *in vivo* by rare earth metals occur only in the case of intravenous injection and not by intramuscular or oral administration, the activity appearing immediately after the injection. As for the behavior of various coagulation factors, the decrease of prothrombin, thrombin, and thrombocytes is marked, while there is no marked change in the amount of fibrinogen, antithrombin, and calcium.

The inhibitive action of rare earth metals *in vivo* is antagonized by vitamin K, which increases the amount of prothrombin, and is synergetic with Dicumarol, which destroys prothrombim. These facts indicate that in this case also, the rare earth metals act on prothrombin in blood stream and retard or inhibit its activation to thrombin.

On the other hand, biological distribution of rare earth metals is most concentrated in the liver tissues. Consecutive intravenous administration of one of the rare earth metals results in damage to liver functions, and patholohistological observations reveal that necrosis and fatty infiltration of liver tissues are most marked. These facts indicate that the rare earth metals possess affinity to the liver, lower the functions of the liver cells, and inhibit the formation of prothrombin in the liver.

Summary

The nature of the inhibition of blood coagulation by rare earth metals *in vitro* is their action on prothrombin in the blood and to inhibit its activation to thrombin. Such action *in vivo* includes, besides the same direct action on blood, their action on the liver cells to inhibit the formation of prothrombin.

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