Nagoya J. med. Sci. 28: 179-195, 1966.

NEW ASPECTS ON THE TREATMENT OF HEMOPHILIA

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ABSTRACT

The therapeutic aspects of hemophilia were studied on a total of 22 cases, consisting of 13 hemophilia A, 8 hemophilia B and one with congenital combined deficiency of Factor V and Factor VIII.

1) The half life of the activity of Factor VIII following intravenous injection of fresh plasma, estimated 7 times in 4 cases, was ranged from 4 to 11 hours. Besides such short half life of Factor VIII, there appeared some cases with acquired resistance to transfusion efficacy after frequently repeated transfusions of blood or its preparation. Therefore blood transfusion should be avoided except when absolutely necessary.

2) Prednisolone and betamethasone were found to possess striking clinical efficacy in all of 9 cases with hemophilia, that was revealed by decrease in frequency of hemorrhage and by accelerated absorption of blood from hemorrhagic foci. But there found no case at all where activity of congenitally deficient clotting factor increased following their administration.

3) Antiplasmic substances, ε-ACA and trans-AMCHA, were also found to have such striking clinical efficacy as corticosteroids. In 15 out of 17 cases with hemophilia were found the decrease in frequency of hemorrhage, and the decrease of pain accompanying hemorrhage in all of 17 cases. Following the administration of antiplasmic substances in these hemophiliacs, the fibrinolytic activity of circulating blood and the inhibitor for formation of intrinsic thromboplastin were decreased, but in no case the activity of congenitally deficient clotting factor increased.

Based on the above results, it is concluded that antiplasmic substances and corticosteroids can be used extremely satisfactorily in the inhibition of hemorrhage in hemophilia.

I. INTRODUCTION

In general hemophilia A is believed to be a disease due to congenital deficiency of Factor VIII, and hemophilia B of Factor IX. And effective treatment of hemophilia has been extremely unsatisfactory. The present paper describes first on blood transfusion therapy that has been employed so far, followed by the results of treatment by corticosteroids and antiplasmic

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Received for publication December 23, 1965.

substances that we have adopted since 1961.

II. MATERIALS AND METHODS

A) A total of 22 cases, consisting of 13 hemophilia A, 8 hemophilia B and one with congenital combined deficiency of Factor V and Factor VIII, were studied. An outline of these cases is indicated in Table 1.

TABLE 1. Tweny-Two Patients with Hemophilia

A) Hemophilia A

Case No.	Age	Onset of hemorrhage (age)	Whole blood clotting time (min)	Factor VIII (%)	Clinical severity
1	9	3	13	3.0	severe
2	4	<1	24	4.6	〃
3	13	<1	28	0.9	〃
4	10	1	55	0.5	〃
5	31	<1	67	0.5	〃
6	7	1	77	0.9	〃
7	45	<1	110	0.2	〃
8	13	<1	120	0.2	〃
9	11	4	131	0.9	〃
10	11	<1	145	0.2	〃
11	10	3	155	0	〃
12	19	1	163	0.2	〃
13	15	2	191	0.6	〃

B) Hemophilia B

Case No.	Age	Onset of hemorrhage (age)	Whole blood clotting time (min)	Factor IX (%)	Clinical severity
14	23	11	21	0.5	moderate
15	26	6	30	0.3	severe
16	15	7	31	0.4	〃
17	10	<1	31	0	〃
18	7	1	66	0.2	〃
19	31	6	180	0	〃
20	7	4	222	0.1	moderate
21	14	4	250	0.4	〃

C) A Case with Combined Deficiency of Factor V and Factor VIII

Case No.	Age	Onset of hemorrhage (age)	Whole blood clotting time (min)	Factor V (%)	Factor VIII (%)	Clinical severity
22	9	2	11	12	8	minimum

B) *Methods of examination*

a) Blood was obtained between 11.00 A.M. and 12.00 noon by the two syringe method. 3.8% sodium citrate solution was used as anticoagulant. To obtain citrated plasma siliconized glassware was used in all cases.

b) The laboratory tests carried out and the metheds employed were as follows:

1) Platelet count: Fonio's method.

2) Whole blood clotting time: Lee-White's method.

3) Recalcification time: 0.2 ml citrated platelet rich plasma plus 0.2 ml 0.025 M $CaCl_2$ with 8×75 mm non-siliconized glass tube.

4) Thromboplastin generation test (TGT): Biggs-Douglas' method[1].

5) Factor VIII: Pitney's method[2].

6) Factor IX: Fukui-Umegaki's method[3].

7) Prothrombin in serum: Stefanini-Crosby's method[4].

8) Fibrinogen: Ratnoff's method[5].

9) Fibrinolytic activity (Euglobulin fraction, Streptokinase-activated euglobulin fraction and Antiplasmin): Fibrin plate method[6].

10) Inhibitor for formation of intrinsic thromboplastin in hemophilic plasma: 0.5 ml each of hemophilic plasma and normal plasma were mixed and the mixture was incubated for 30 minutes at 37°C, at the end of which time it was adsorbed by 200 mg of barium sulphate. The TGT was then conducted with this adsorbed plasma, normal serum and phospholipid. In parallel with the above procedure a control test with 0.85% saline instead of hemophilic plasma was conducted by an identical procedure. By comparing these two thromboplastic activities the grade of fall in activity of intrinsic thromboplastin formation of normal plasma induced by hemophilic plasma was computed. This grade of fall was taken to represent the activity of the inhibitor.

11) Concentration of ε-aminocaproic acid (ε-ACA) in the blood; ε-ACA was extracted from 0.5 ml of serum containing ε-ACA with 9.5 ml of absolute ethanol. The pasty residual obtained by evaporation of this ethanol fraction containing ε-ACA on a water bath was dissolved in 0.25 ml of distilled water. The solution was then subjected to paperchromatography, with N-butanol—acetic acid—distilled water (4 : 1 : 4) as solvent. After 12 hours coloration was induced by ninhydrin. Of the spots developed that of Rf 0.6 was extracted with N/100 NaOH. The concentration of ε-ACA in this extracted fluid was then estimated with a Beckman spectrophotometer at 570 mμ.

III. CLASSIFICATION OF THE SEVERITY OF HEMOPHILIACS

From the findings that the clinical manifestations of hemophiliacs do not always run parallel with the data of coagulation tests, we classified hemophiliacs into three groups according to severity of clinical symptome based on hemorrhagic manifestations, and unrelated to the data of coagulation test. The "severe group" consists of cases with hemorrhagic manifestations occurring on more than half of the days of a month and is characterized by severe disturbance of daily activities. The "minimum group" presents only abnormal hemorrhagic tendencies following external injuries or surgical operations and can maintain a normal daily life. The "moderate group" lies in between the two, stated above, as regards severity of clinical manifestations. The subjects

studied here consisted of 18 cases of severe group, 3 of moderate group and one of minimum group, as shown in Table 1.

IV. SOME PROBLEMS ON BLOOD TRANSFUSION THERAPY

Transfusion of blood, plasma or Factor VIII preparations are widely known to be indispensable in the treatment of hemophiliacs, but there are some problems difficult to solve in this treatment, such as those stated below:

i) The half life of the activity of Factor VIII following intravenous injection of fresh plasma was estimated 7 times in 4 cases consisting of 3 hemophilia A (cases Nos. 6, 7, 13) and one of congenital combined deficiency of Eactor V and Factor VIII (case No. 22), when it was found that the half life of Factor VIII activity lay between 4 to 11 hours, as indicated in Table 2. Hence, in order to maintain the activity of Factor VIII above a definite level it becomes necessary to repeat fresh plasma transfusions in succession.

TABLE 2. Turnover Rate of Factor VIII

Case No.	Body weight (kg)	Transfused plasma volume (ml)	Activity of Factor VIII		Half life of Factor VIII (hours)
			Before transfusion (%)	30 min after transfusion (%)	
6	26	400	0.1	7.0	4
7	54	400	0.1	9.8	11
		400	0.1	11.0	9
13	28	200	0.3	5.6	8
22	28	400	9.0	40.0	4
		400	10.0	50.0	4
		400	8.0	36.0	4

ii) There are cases where the efficacy of blood transfusions gradually falls when repeated several times. Fig. 1 shows a case (case No. 19) with such a tendency. As may be seen from the figure, following the first transfusion of lyophilized fresh plasma the whole blood clotting time which had once shortened gradually became longer and took 7 days for it to exceed 30 minutes. However, following the second transfusion the period for the whole blood clotting time to exceed 30 minutes became 4 days, and following the third transfusion 2 days, indicating gradual shortening of the above time.

iii) Again, though blood transfusions were found to be effective during early childhood we found in case No. 10 that today, at age 11, due to the presence of a powerful inhibitor for intrinsic thromboplastin formation in the blood, large volume transfusions of fresh blood can no longer shorten the whole blood clotting time. Table 3 shows the results of cross-match-tests with

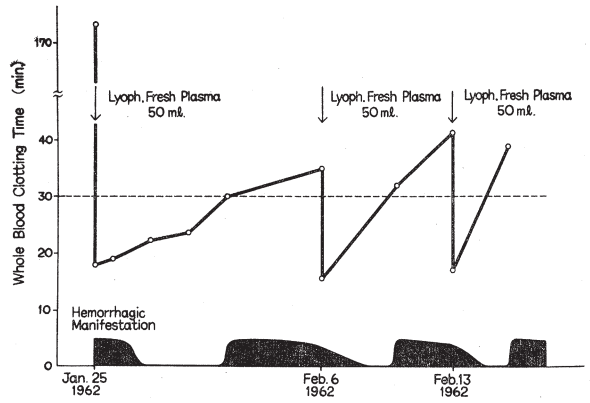


FIG. 1. Responses to lyophilized fresh plasma in a case with hemophilia B (Case No. 19).

TABLE 3. Results of Cross-Match-Tests between Case No. 10 and Normal Subject

Mixing ratio of whole blood or plasma (%) (patient : normal)	Whole blood clotting time (min)	Recaldification time (sec)	Activity of adsorbed plasma in TGT (%)
100 : 1	145	2200	9
90 : 10	17	2200	9
75 : 25	13	2000	9
50 : 50	8	1700	9
25 : 75	8	400	18
0 : 100	8	120	100

blood of this case and normal blood. It will be seen that the TGT conducted at the end of 30 minutes' incubation at 37°C of the mixture of patient's plasma with normal plasma, showed one volume of patient plasma to almost completely inhibit the intrinsic thromboplasitn formation of three volume of normal plasma. However, when preincubation was not conducted and the cross-match-test with whole blood clotting time was made immediately following

TABLE 4. Physicochemical Properties of Inhibitor for Formation of Intrinsic Thromboplastin in Hemophilia

	Case No. 10	Bigge (1961)	Deutsch (1961)
plasma	+	+	+
serum	—		—
adsorption on $BaSO_4$	—	—	—
heat-stability 56°C, 10 min	stable	stable	stable
65°C, 10 min	stable		stable
storage-stability 4°C	stable	stable	stable
−20°C	stable	stable	stable
precipitation with saturated $(NH_4)_2SO_4$	0-50%	33-50%	25-50%
dialysis	—	—	—

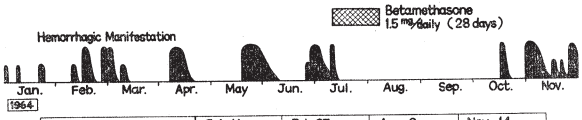
mixture of patient blood with normal blood, such powerful inhibition could not be demonstrated. Thus, this inhibitor reaction is of the slow type, and its nature is somewhat different from the immune antibody usually encountered. This inhibitor is acquired in nature and is believed to result from repeated blood transfusions. Table 4 shows the characteristics of the inhibitor of this case, compared with those previously reported[7) 8)].

V. ON THE CORTICOSTEROIDS THERAPY

Reports on the clinical use of corticosteroids for treatment of hemophilia have been extremely rare. In the present work we studied the efficacy of this therapy in 9 cases, consisting of 4 of hemophilia A (cases Nos. 5, 6, 7, 9) and 5 of hemophilia B (Case Nos. 14, 17, 19, 20, 21).

i) Case reports

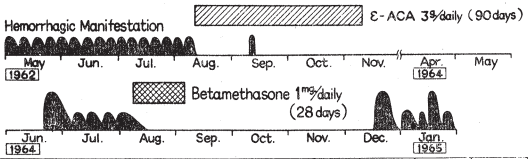
Case No. 9; hemophilia A, aged 11, male (Fig. 2): Characterized by frequent occurrence of severe hemarthrosis in the shoulder, elbow and knee joints, rendering normal daily activities almost impossible. 1.5 mg/daily of betamethasone was given orally for 28 days. Hemorrhagic manifestations improved conspicuously and for three months thereafter no hemorrhages occurred. Following administration of betamethasone practically no change was noted in whole blood clotting time, intrinsic thromboplastin activity and Factor VIII activity, but in the fibrinolytic system euglobulin activity rapidly fell, from 173 MSD units/ml before administration to zero. Again, antiplasmic activity gradually rose, from 7100 MSD units/ml to 13000 MSD units/ml.



		Jul. 11 1964	Jul. 25 1964	Aug. 8 1964	Nov. 14 1964
Treatment		(—)	Betamethasone 1.5 mg/d. (14 days)	Betamethasone 1.5 mg/d. (28 days)	(—)
Hemorrhagic Manifestation		Hemarthrosis	(—)	(—)	Hemarthrosis
Whole Blood Clotting Time (min.)		14.0	14.0	14.0	15.0
TGT (ads. Plasma) (%)		6	3	2	6
Factor VIII (%)		0.1	0	0	0
Fibrinolytic Activity (MSD u/ml.)	Euglobulin	173	0	0	0
	SK-Euglobulin	820	1,200	1,600	2,600
	Antiplasmin	7,100	8,600	12,900	13,000

FIG. 2. Hemophilia A, age 11, male (Case No. 9).

Case No. 6; hemophilia A, aged 7, male (Fig. 3): Due to frequent joint hemorrhages walking became impossible. 1.0 mg/daily of betamethasone was given for 28 days, resulting in absence of hemorrhages for 4 months thereafter. Following administration of betamethasone the whole blood clotting time de-



		Aug. 11 1962	Sep. 8 1962	Oct. 13 1962	Aug. 13 1964	Aug. 22 1964	Sep. 5 1964	Jan. 30 1965
Treatment		(—)	ε-ACA 3%d(28days)	ε-ACA 3%(63days)	(—)	Betamethasone 1mg%(14days)	Betamethasone 1mg%(28days)	(—)
Hemorrhagic Manifestation		Hemarthrosis (+)	(—)	(—)	Hemarthrosis (+)	(—)	(—)	Hemarthrosis
Whole Blood Clotting Time (min)		77	105	123	120	75	85	185
TGT (ads. Plasma) (%)		6	8	12	2	3	4	10
Factor VIII (%)		0.8	0.2	0.4	0	0	0	0
Fibrinolytic Activity (MSD u/ml)	Euglobulin	0	0	0	0	0		0
	SK-Euglobulin	370	4,000	1,220	1,240	400		3,800
	Antiplasmin	6,000	9,700	6,800	5,600	11,000		13,500

FIG. 3. Hemophilia A, age 7, male (Case No. 6)

creased from 120 minutes before administration to 75–85 minutes, but increased again to 185 minutes with the completion of therapy. There was seen no change at all in Factor VIII activity. In the fibrinolytic system, there was seen, as in the previous case, a gradual rise of antiplasmic activity, from 5600 MSD units/ml before administration to 13500 MSD units/ml.

ii) In Table 5 are summarised the clinical efficacies of corticosteroids. As corticosteroids prednisolone in a daily dose of 20–30 mg (cases Nos. 14, 19) or betamethasone 1–2 mg daily (cases Nos. 5, 6, 7, 9, 17, 20, 21) was given orally for 4–6 weeks.

Clinical efficacy was revealed by decrease in frequency of hemorrhage in the severe group, and by accelerated absorption of hemorrhagic joint swelling

TABLE 5. Effects of Corticosteroids in Hemophilia

Case No.	Type of hemophilia	Activities of F. VIII or F. IX (%)	Clinical severity	Corticosteroids		Effect of corticosteroids	
				Daily doses (mg)	Duration (weeks)	Acceleration of absorption of hemorrhages**	Decreasings of number of hemorrhagic manifestation***
5	A	0.5	severe	2.0(B)*	4	+	+
6	A	0	〃	1.0(B)	4	+	++
7	A	0.2	〃	2.0(B)	4	+	+
9	A	0.1	〃	1.5(B)	4	+	++
14	B	0.5	moderate	20 (P)	4	+	
17	B	0	severe	1.0(B)	6	+	+
19	B	0	〃	30 (P)	6	++	+
20	B	0.1	moderate	1.0(B)	4	—	
21	B	0.4	〃	2.0(B)	6	++	

*; (B) indicates Betamethasone and (P) indicates Prednisolone, respectively.
**; The "hemorrhage" indicate hemarthrosis, hematoma and intramuscular hemorrhage.
***; The "decreasing of numbers of hemorrhagic manifestation" was very difficult to judge in the moderate group, so that no data were given in this group.

and/or intramuscular hemorrhage in 8 out of 9 cases.

Hemostatic tests showed shortening of whole blood clotting time in 4 cases (cases Nos. 6, 17, 20, 21) following corticosteroids administration, but practically no change was recognized in intrinsic thromboplastin activity and Factor VIII or Factor IX activity. In the fibrinolytic system, there were noted decrease of activity in 2 cases (cases Nos. 9, 21), and increase of antiplasmic activity in 3 cases (cases Nos. 6, 9, 14), but no change was seen in SK-activated euglobulin activity.

iii) Corticosteroids also promote the absorption of blood from hemorrhagic foci and thereby help to prevent ankylosis to occur. Table 6 describes such a case.

TABLE 6. Effect of Corticosteroid on Improvement of Joint-Limitation
—Hemophilia B, Case No. 21—

	1964 18, Jul.	〃 1, Aug.	〃 15, Aug.	〃 29, Aug.
total doses of betamethasone (2 mg/daily)	0	28 mg	56 mg	84 mg
range of movement {left hip-joint	10°	35°	90°	90°
range of movement {left knee-joint	40°	40°	120°	120°
whole blood clotting time (min)	250	150	65	75
adsorbed plasma activity (TGT, %)	105	80	103	120
serum activity (TGT, %)	3	4	5	7
factor IX (%)	0.4	0.2	0	0.2
fibrinolytic activity (MSD units/ml)				
euglobulin	94	0	0	
SK-activated euglobulin	1700	3400	1500	
antiplasmin	10600	12500	12800	

Case No. 21; hemophilia B, aged 14, male (Table 6): The patient complained of limitation of the left hip joint and left knee joint, namely, the range of movement of the hip being 10° and of the knee 40°. And the administration of 2 mg daily of betamethasone was commenced, resulting in conspicuous improvement in the limitation of the joints, the range of movement of the hip recovering to 90° and of the knee to 120° at the end of 28-day administration.

iv) Thus, corticosteroids were found to possess striking clinical efficacy in hemophilia, but the prolonged administration can not be recommended because of the side effects of this drug.

VI. ON THE ANTIPLASMIC SUBSTANCES THERAPY

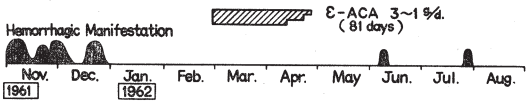
In 1961 we[9] and Abe[10] first employed antiplasmic substance in the management of bleeding in hemophilia. Below are described the effects of this treat ment in hemophilia, using the antiplasmic substances, ε-aminocaproic acid (ε-

ACA) and trans-1-aminomethyl-cyclohexane-4-carboxylic acid (trans-AMCHA).

The subjects treated totaled 17, consisting of 12 of hemophilia A (cases Nos. 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13) and 5 of hemophilia B (cases Nos. 15, 16, 17, 18, 19).

i) Case reports

Case No. 1; hemophilia A, aged 9, male (Fig. 4): Hemorrhagic manifestations occurred only 1-2 times a month, but 15-30 days were needed for the absorption of the exuded blood, so that normal daily activity was greatly hindered. When 3 g daily of ε-ACA was administered orally hemorrhagic manifestations disappeared completely. Treatment was ended when 211 g were given for 83 days. Since then 3 years have passed but hemorrhagic manifestations remained improved except for slight grade of purpura occurring at sites of trauma once in 1-2 months. The patient is being without further treatment. Hemostatic tests revealed practically no change in the blood clotting system and fibrinolytic system, as shown in the figure.



		Dec. 23 1961	Jan. 13 1962	Jan. 18 1962
Treatment		(−)	ε-ACA 3g/d. (7days)	ε-ACA 3g/d. (120days)
Hemorrhagic Manifestation		Hemarthrosis	(−)	(−)
Whole Blood Clotting time (min)		13	16	13
TGT (ads.Plasma) (%)		32	32	22
Factor Ⅷ (%)		3	2	2
Fibrinolytic Activity (MSD γ/mL.)	Euglobulin	0	0	9
	SK-Euglobulin	1440	500	390
	Antiplasmin	8200	5500	6600

FIG. 4. Hemophilia A, age 9, male (Case No. 1)

Case No. 15, hemophilia B, aged 26, male (Fig. 5): In this case the complaints of frequent hemarthrosis and intramuscular hemorrhages were seen. In December 1961 treatment of 6 g daily of ε-ACA orally was commenced, resulting striking improvement of the hemorrhagic manifestation and ability to lead a normal daily life. ε-ACA was administered for 12 months, totalling 2040 g. The patient's condition has remained satisfactory without further treatment, but in January 1964 hemorrhagic manifestations reappeared. However, on administering 500 mg daily of trans-AMCHA orally there resulted as with ε-ACA rapid improvement in the hemorrhagic manifestations. But when therapy was interrupted hemorrhages reappeared, so that treatment with trans-AMCHA is still being continued.

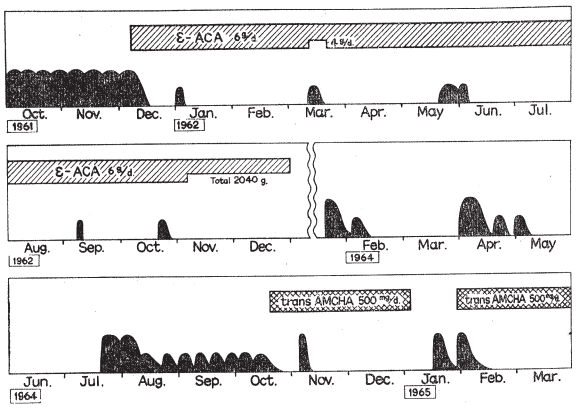


FIG. 5. Hemophilia B, age 26, male (Case No. 15)

Case No. 3; hemophilia A, aged 13, male (Fig. 6): In this case marked improvement occurred with ε-ACA therapy likewise, but when treatment was interrupted or the dose decreased there was noted tendency for hemorrhagic manifestations to become worse. Hence, treatment is still being continued, for 3 years and 10 months in doses of 3–6 g daily.

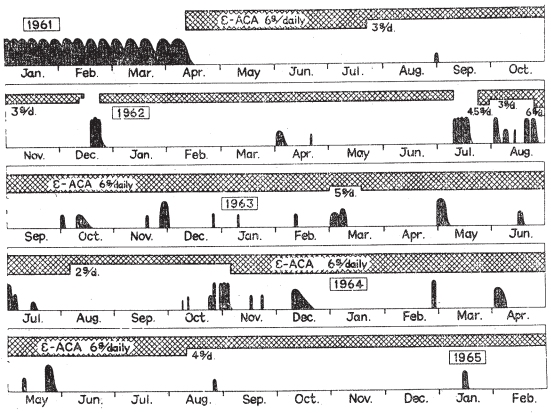


FIG. 6. Hemophilia A, age 13, male (Case No. 3)

ii) In Table 7 are summarized the clinical efficacies of antiplasmic substances. Cases Nos. 1, 2, 3, 4, 5, 6, 9, 15, 17 were characterized by more than 3 weeks with bleeding a month before therapy, which decreased to only a few days after therapy. These 9 cases constitute the strikingly effective group. In 6 cases (cases Nos. 11, 12, 13, 16, 18, 19) the days with bleeding a month decreased to one-third to one-half after therapy, and these constitute the effective group. Case Nos. 8, 10 showed no improvement, and were therefore classified as the non-effective group. In all cases the pain accompanying hemor-

TABLE 7. Effects of Antiplasmic Substances in Hemophilia

Case No.	Duration of administraton of antiplasmic substances* (months)	Effects of antiplasmic substances				
		Days with hemorrhage per month (days)		Improvement of hemorrhage	Improvement of pain at hemarthrosis	Effectiveness
		Before administration	During administration			
1	3	>21	0			markedly effective
4	3	〃	1 3	++	+	〃
15	12	〃	1.5	++	++	〃
9	4	〃	1.5	++	++	〃
6	6	〃	1.8	++	+	〃
5	5	〃	2.6	++	+	〃
17	25	〃	5.5	+	+	〃
2	9	〃	6.0	++	+	〃
3	46	〃	6.0	+	+	〃
16	16	〃	7.3	+	+	effective
13	16	〃	9.2	+	+	〃
18	19	〃	9.3	+	++	〃
19	22	〃	9.7	−	+	〃
12	19	〃	9.8	+	++	〃
11	8	〃	13.1	+	+	〃
8	14	〃	19.3	−	+	non-effective
10	14	〃	31.0	−	+	〃

*; Antiplasmic substances were administered orally in following daily doses; 3-8 g of ε-ACA and 500-750 mg of trans-AMCHA, respectively.

rhage decreased. Of these cases in four (cases Nos. 1, 5, 9, 13) the days of bleeding in a month were less than about one week during the year left untreated following completion of therapy with antiplasmic substances, indicating maintainance of a satisfactory state with improvement in hemorrhagic manifestations.

In these 17 cases treated no side effect was recognized at all.

iii) The daily doses of antiplasmic substances administered were in case of ε-ACA 100-200 mg/kg and in case of trans-AMCHA 10-20 mg/kg. They were given orally, and the doses adjusted at will for each case. However, attention has to be paid to the following experimented findings. Fig. 7 shows the results of chromatography conducted to determine the fluctuations in concentration in the blood following intravenous injection of 40 mg/kg of ε-ACA. The oblique line area represents the normal range. As the figure indicates, in hemophiliacs the speed of disappearance of ε-ACA from the circulating blood during the non-bleeding phases is practically similar to that of the normal controls, but during the bleeding phases the speed is considerably accelerated. Hence, during the hemorrhagic phases the need arises to administer ε-ACA in larger doses and more frequently than during the non-hemorrhagic phases.

iv) The changes in the blood clotting system and factors of the fibrinolytic system following administration of antiplasmic substances have been studied.

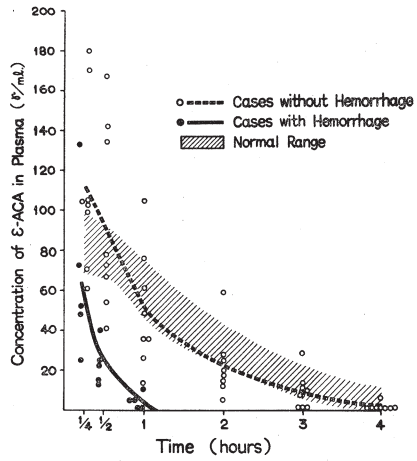


FIG. 7. The disappearance of intravenously administered ε-ACA from the plasma of hemophiliacs.

a) The administration of antiplasmic substances caused some changes in whole blood clotting time (Table 8) and activities of Factor VIII and IX (Table 9), but no definite tendency was recognized. In contrast the fibrinolytic system showed significant changes (Table 10), namely after 7 months there was noted a marked decrease in number of tests with increased fibrinolytic activity in the "strikingly effective group", but not so marked change was found in the "non-effective" and "effective" group. However, in the strikingly effective group when marked clinical efficacy was demonstrable during the

TABLE 8. Changes in the Whole Blood Clotting Time in Hemophilia during Administration of Antiplasmic Substances

Case No.	Duration of Administration of Antiplasmic Substances (month)						
	0	1	2	3	4-6	7-9	10-12
	Whole Blood Clotting time (min)						
1	13	16					
2	24	15	28	28			
3	28	28		30	20	21	28
4	55	39		34	93		
5	67	70	57	85			
6	77	105	123				
8	120	190	165	170	172	137	165
9	131	87	133	140			
10	190	120	100	88	120	115	167
12	163	210					150
13	191	210	157		220	235	200
15	30	21	30	27	50	20	35
16	31	35	24		32	45	37
17	31	23	28		70	64	36
18	66	280	310		240	300	270
19	180	180	200		140	225	220

TABLE 9. Changes in the Activities of Factor VIII and Factor IX in Hemophilia during Administration of Antiplasmic Substances

Case No.	Duration of Administration of Antiplasmic Substances (month)						
	0	1	2	3	4-6	7-9	10-12
	Activities of Factor VIII or Factor IX (%)*						
2	4.6	4.2		7.0			
3	0 9		0.3		0.5	0.4	0.6
4	0.5	0	0.9	0.2			
5	0.5	0.1	0.4	0.6			
6	0.9	0.2	0.4	0			
8	0.2	0.7	0.3	0.5	0.1	0	0
9	0.9	0	0	0.4			
10	0.2	0.9	0.2	0.8	0.1	0	0.2
11	0	0	0		0.6		
13	0.6	0	0.6		0	0.4	0.3
15	5.0	6.0	6.0	5.2	1.3	6.5	2.0
16	0.4	0.1	0.7		0.2		0.4
17	2.0	2.0	1.9	2.2	1.8	1.8	1.6
18	1.0	0.8	0.2		0.6	0.4	0.2
19	0.4	0.4	0.3		0.3	0.3	1.0

* The data show the activities of congenitally deficient clottingfactor; namely, Factor VIII in hemophilia A (case Nos. 2, 3, 4, 5, 6, 8, 9, 10, 11, 13) and Factor IX in hemophilia B (case Nos. 15, 16, 17, 18, 19).

TABLE 10. Changes in Fibrinolytic Activity in Hemophilia during Administration of Antiplasmic Substances

	Duration of Administration of Antiplasmic Substances		
	0	1-6	7-12
	Number of Tests with Increased Fibrinolytic Activity* / Total Number of Tests		
markedly effective group (9 patients)	6/9 (64%)	14/22(64%)	3/12(25%)
effective group (6 patients)	5/6 (83%)	10/14(71%)	7/11(64%)
non-effective group (2 patients)	2/2(100%)	10/14(71%)	6/ 8(75%)

* "Tests with increased fibrinolytic activity" indicate the cases who correspond to at least one of the following three items;

1) The cases whose fibrinolytic activity of euglobulin are more than 70 MSD units/ml.
2) The cases whose fibrinolytic activity of SK-activated euglobulin are more than 2400 MSD units/ml.
3) The cases whose antiplasmic activity are less than 6000 MSD units/ml.

period 1-6 months after commencement of the therapy, the findings of the fibrinolytic system showed not much changes as compared with that before commencement of therapy, so that the variations in fibrinolytic activity seen in the circulating blood can not wholly account for the clinical efficacy of antiplasmic substances.

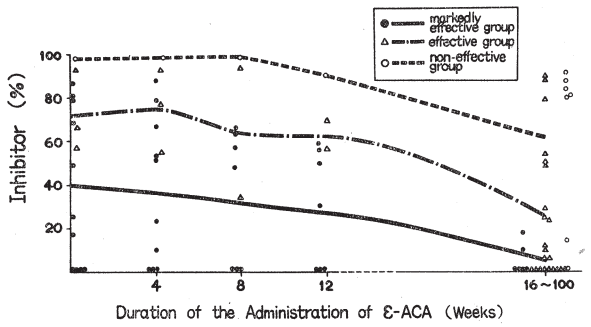


FIG. 8. The therapeutic effect of ε-ACA on the inhibitor for formation of intrinsic thromboplastin in hemophiliacs.

b) Regarding the effect of inhibition of normal thromboplastin formation present in the blood of hemophiliacs, there have been many reports. Fig. 8 shows the relation of this inhibitor for formation of intrinsic thromboplastin to antiplasmic substances. It will be seen that in all three groups of "strikingly effective", "effective" and "non-effective" there occurred gradual decrease of this inhibitor, and in the first group it became ultimately zero but in the "non-effective" group the content fell to about 66% and continued to remain so. Thus, it became clear that this inhibitor is influenced by the administration of ε-ACA, and this phenomenon is believed to be related in part with the clinical efficacy noted following administration of ε-ACA.

VII. DISCUSSION

The effect of the transfusion of the blood and blood preparations in hemophilia is well recognized, and today such a transfusion has become an indispensable measure in the treatment of severe hemorrhages in hemophilia and during surgical operations. However, our findings regarding the half-life of Factor VIII revealed it to be only 4-11 hours, and previous investigations by others have also shown it to be short; namely, 4 hours (Brinkhous[11], 1956), 9 hours (Douglas[12], 1958), 3-4 hours (Van Creveld[13], 1959) and 7-22 hours with the mean of 14 hours (Biggs[14], 1963). Regarding the half-life of Factor IX there are only a few reports[14)15)16)17)] available, with the values ranging from 18 to 72 hours and being indefinite. Again, as regards the minimum level of Factor VIII needed to raise artificially for it to act as a hemostatic has been reported to be 5% by Brinkhous[11] (1956), 10-20% by Rosenthal[18] (1957), 30% by Pitney[19] (1957), 30% by Macfarlane[20] (1957), 30% by Blomböck and Nilsson[21] (1958), 5-10% by Ratnoff[22] (1960) and 5-10% by Yoshida[23] (1960). Levels higher than the above are said to be necessary at the time of surgical operation. Hence, in hemophilia A large and frequent blood transfusions have to be repeated in order to maintain an effective Factor VIII level.

Another problem that has to be studied in transfusion therapy is the appearance of resistance to transfusion efficacy, namely the formation of acquired circulating anticoagulant. Regarding the existence of an inhibitor to Factor VIII in the circulating blood of hemophiliacs there have appeared many reports since the work of Laurence and Johnson[24] (1941) on this substance. Practically all are concerned with cases where enormous and frequent blood or its preparations were employed, and the frequency of occurrence varies with each investigater. According to Ratnoff[22] (1960) this inhibitor is recognized in 20% or more of all hemophiliacs, but Biggs[7] (1962) reported it to exist in only 1-3% of severe cases, and Ikkala[25] (1960) in only one of 122 cases (0.8%). However, all agree that this inhibitor is found only in grave cases of hemophilia, and also that it is acquired by frequent transfusions of blood or its preparations in large quantities. Regarding these problems it is of interest that van Creveld[26] (1949) noted the formation of this inhibitor to be extremely poor when heparinized plasma is used instead of citrated plasma.

In view of the above findings, it will be clear that blood transfusions should be avoided except when absolutely necessary.

Regarding the efficacy of corticosteroids in hemophilia Ozsoylu[27] *et al.* (1962) stated that the antihemophilic-factor-level increases considerably in moderately or mildly affected classic hemophiliacs under treatment with high doses of corticosteroids, but no statement was made of their clinical efficacy. Künzer[28] (1964) on the one hand, denied their efficacy both clinically and from the aspect of coagulation study. Trieger[29] (1962), however, noted the administration of 80 mg daily of prednisone to be effective in decreasing the amount of blood transfusion necessary when undertaking tooth extractions in hemophiliacs, suggesting the value of corticosteroids in hemophilliacs. As has been stated already we recognized the striking clinical efficacy of corticosteroids in hemophilia, but there were found no case at all where Factor VIII (in hemophilia A) and Factor IX (in hemophilia B) increased following their administration. With other hemostatic tests there were noted only 4 cases with shortening of whole blood clotting time, only 2 cases showing lowering of fibrinolytic activity of the euglobulin fraction and only 3 with increase of antiplasmic activity. These results failed to explain therefore the striking clinical efficacy already described.

The inhibitory effects of the antiplasmic substance, ε-ACA, on the hemorrhagic manifestations of hemophilia were first reported by us[9] (1962) and Abe *et al*[10]. (1962), and today ε-ACA and trans-AMCHA are being widely used in the long term control of hemorrhage in hemophilia. Recently, Reid[30] (1964) reported that with a daily dose of 12-40 g of ε-ACA administered to 11 cases of hemophiliacs needing teeth extraction, there arose no need at all for blood transfusion. On the other hand Nour-Eldin[31] (1962) has denied the efficacy of ε-ACA in hemophilia, but as has been stated above we obtained extremely

satisfactory resuls.

As regarding the action mechanism of antiplasmic substances against hemophilia the inhibition of fibrinolytic activity of the circulating blood and decrease in amount of the inhibitor for formation of intrinsic thromboplastin can be considered, but these alone can not explain all aspects of the action mechanism, and further studies are probably necessary.

In cases No. 5, 6, 9, 17, 19 where corticosteroids were employed in combination with antiplasmic substances, it was interesting to note that the inhibitory effects of the two on the hemorrhagic manifestations ran parallel, and it seems that further studies on the mechanism of action should be attempted based on the relation of these two substances.

VIII. CONCLUSION

In the clinical treatment of hemophilia blood transfusions are a must at the times of massive hemorrhage and surgical operations. However antiplasmic substances and corticosteroids can also be used extremely satisfactorily in the inhibition of hemorrhage in hemophilia. From the findings that side-effects are very mild and the efficacy very high, antiplasmic substances are of value in the long term control of hemorrhagic manifestations of the hemophilia.

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