in pigeons with depression in body weight and feed intake. No level of reserpine was found which would inhibit TSR without depressing feed intake. It is suggested that depression of thyroid activity at higher levels of reserpine is secondary to depression in food intake.

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Factor VIII (Antihemophilic Factor) and Factor V (Proaccelerin) Activity of Platelets.* (25819)

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Roskam postulated that normal platelets contain on their surface plasma coagulation factors. He used the expression "plasmatic atmosphere of platelets" to describe this phenomenon(1). Mann *et al.*(2) and later Ware *et al.*(3) showed that normal platelets have factor V activity. Experiments of Hjort *et al.* (4) supported the theory that factor V was adsorbed on the platelet surface. Bounameaux(5) demonstrated the multiplicity of plasma factors adsorbed on platelet surface. The present paper is concerned with 1) demonstrating that normal platelets contain material with factor VIII or factor V activity in quantities sufficient to correct to normal certain diagnostic tests for study of blood from patients with defects of these factors; 2) demonstrating that platelets separated from blood of patients with plasma deficiency of factor VIII or factor V present a defective activity of the corresponding factor; 3) describing some properties of these active factors of platelets.

Materials and methods. Materials and methods used for diagnostic purposes have been described(6,7). All plasma samples used were obtained by mixing blood with 3.8% sodium citrate in proportion of 1:9. Platelets were separated by differential centrifugation and washed 10 times with normal saline. Concentration of normal platelet suspension was then made equal to that of patient's platelets, following a semivolumetric method previously described(6). A 1% suspension v/v was used unless otherwise indicated. When lyophilized platelets were used, weight of dry material was equivalent to that of fresh elements contained in 1% concentration. Platelet suspensions were used in various coagulation mixtures according to technic of "thromboplastin generation test" (TGT), described by Biggs and Douglas(8). Our results are, however, reported in 1 figure (called "prothrombin activation index" or PAI)(6) rather than with a curve. Normal PAI is 9.8 $\pm$ 2.9 (2 standard deviations). The systems used are described with specific experiments. Other coagulation mixtures were tested following the basic technic of "prothrombin time" (one-stage method). Systems used are described with specific experiments. Tissue thromboplastin was a commercial preparation obtained from rabbit brain.†

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TABLE I. Factor VIII Activity of Normal Platelets and Absence of Activity of Platelets from 2 Hemophilics and a Patient with Acquired Factor VIII Deficiency. System activated by calcium ions (M/40 $CaCl_2$) and activity estimated on normal substrate.

Plasma	Serum	Platelets	PAI
N	N	N	9.4
H^1	H^1	H^1	38.0
H^2	H^2	H^2	42.1
H^1	H^1	N	11.7
H^1	H^1	H^2	43.2
N	N	H^1	9.6
A	A	A	40.8
A	A	H^1	36.2
A	A	N	12.4

N = Normal. H = Hemophilics. A = Acquired AHF deficiency.

Results. I) *Factor VIII activity of platelets.* 1) Materials for this experiment were Al(OH)-treated plasma diluted 1:5 with normal saline, serum diluted 1:10 with normal saline and platelets. These materials were obtained from a normal subject, 2 hemophilics, and a 62-year-old white man with acquired factor VIII deficiency, and used immediately after preparation. Criteria for diagnosis of classical hemophilia were hemorrhagic diathesis, positive family history, plasma deficiency demonstrable with TGT and absence of correction of plasma defect by mixture with plasma of a known hemophilic. Criteria for diagnosis of "acquired factor VIII deficiency" were severe hemorrhagic diathesis with onset in old age, absence of family history, plasma defect demonstrable with TGT, absence of correction with known hemophilic plasma, abnormal electrophoretic pattern, clinical and laboratory evidence of remissions on steroid therapy associated with correction of electrophoretic pattern to normal. The above materials were variously combined and activity of mixtures estimated on normal substrate. A representative experiment is shown in Table I.

TABLE II. Factor VIII Activity of Platelets Immediately after Separation, after 6 Months of Storage, after Lyophilization and after 24-Hr Incubation at 37°C. Estimation of activity as in Table I.

Plasma	Serum	Platelets	PAI
N	N	N	9.8
H	H	H	42.0
H	H	N O	16.0
H	H	N L	23.0
H I	H I	N	10.9
N	N	N I	18.0

H = Hemophilic. N = Normal. I = Incubated. O = Aged. L = Lyophilized.

2) Materials for this experiment were 6-month-old platelets separated from normal blood and kept in plastic containers suspended in small amount of plasma; platelets lyophilized after separation from normal blood, triple washing with saline and suspension in normal saline. The dry material was kept at room temperature for approximately one year; Al(OH)-treated plasma diluted 1:5 with normal saline; serum diluted 1:10 with normal saline and platelets from normal and hemophilic subjects used immediately after separation; plasmas incubated 24 hours at 37°C, then adsorbed with Al(OH) and diluted 1:5 with normal saline; hemophilic serum incubated at 37°C for 24 hours, then diluted 1:10 with normal saline; normal platelets incubated at 37°C for 24 hours. A representative experiment is shown in Table II.

TABLE III. Factor V Activity of Normal Platelets and Absence of Activity of Platelets from a Patient with Congenital Factor V Deficiency. One-stage method. System activated by tissue thromboplastin and calcium ions (0.02 M $CaCl_2$).

Patient's plasma	Patient's platelets	Normal platelets	Normal saline	Clotting time, sec.
.2				62
.1			.1	70
.1	.1			72
.1		.1		25

II) *Factor V activity of platelets.* 1) Materials in this experiment were plasma and platelets from normal individuals and from a patient with deficiency of factor V. This patient has been described(9). Materials were used immediately after separation. A representative experiment is shown in Table III. 2) Various mixtures were prepared using normal plasma, factor V-deficient plasma, normal platelets and platelets from patient with factor V deficiency. The mixtures were in contact for 18 hours at refrigerator temperature, then platelets were separated by centrifugation and washed 10 times with normal saline. Activity of incubated platelets was estimated by one-stage method using fresh factor V-deficient plasma (obtained from patient men-

tioned above) as substrate. Results are shown in Table IV.

3) Materials were hemophilic plasma adsorbed with Al(OH) and diluted 1:5 with normal saline either immediately after separation or after 24-hour incubation at 37°C; normal or hemophilic serum diluted 1:10 with saline; platelets separated from normal plasma or factor V-deficient plasma, and used immediately after 10 washings. Hemophilic plasma incubated 24 hours showed "prothrombin time" of 16 seconds and contained less than 5% of factor V activity. Plasma from factor V-deficient patient did not correct this abnormality. The materials were variously combined in TGT systems. Results of representative experiments are shown in Table V.

Comments and conclusions. Serum of hemophilic patients contains larger amounts of prothrombin than normal serum. Elevated serum prothrombin is probably responsible for an apparently smaller deficiency observed when TGT is performed using hemophilic plasma, serum and phospholipidic compounds, as compared with that observed when normal serum is substituted in that system for hemophilic serum. Elevated prothrombin activity of hemophilic serum undoubtedly contributed to reduction of PAI; however, maintaining the system "hemophilic plasma-hemophilic serum" unchanged, values of PAI are much greater when hemophilic platelets are added than when normal platelets are used (Table I). Defective activity of platelets was not characteristic of a single patient nor was it confined to congenital conditions. As expected, hemophilic platelets added to normal plasma and serum showed no abnormality (Table I). It is concluded that activity of platelets is of plasmatic origin. The active factor cannot be completely removed by multiple washings, is stable in stored platelets and in lyophilized material, but appears heat labile (Table II).

TABLE IV. Activity of Platelets from Normal Individual and from Patient with Congenital Factor V Deficiency after Prolonged Contact with Normal Plasma and Factor V-Deficient Plasma. Patient's platelets are devoid of factor V activity but become active after contact with normal plasma. Normal platelets lose their activity after contact with factor V-deficient plasma.

Patient's plasma	Saline	.1 platelet preparation	Clotting time, sec.
.2			67
.1	.1		74
.1		Pp	65
.1		Pn	25
.1		Np	42
.1		Nn	21

N = Normal. P = Factor V-deficient. Small letters indicate source of plasma with which platelets were kept in contact.

TABLE V. Factor VIII Activity of Platelets from a Factor V-Deficient Patient Is Intact. After incubation of hemophilic plasma, factor V was destroyed and platelets from the factor V-deficient patients no longer activate the system. Estimation of activity as in Table I.

Plasma	Serum	Platelets	PAI
N	N	N	9.2
H	H	H	31.2
H	H	N	10.4
H	H	P	13.0
H I	H	N	19.3
H I	H	P	44.6

N = Normal. H = Hemophilic. I = Incubated. P = Factor V-deficient.

Normal platelets demonstrated also factor V activity. Platelets from a patient with factor V deficiency lacked this activity (Table III), but contact with normal plasma resulted in acquisition of activity of previously defective elements. This was not observed when platelets and plasma from the same patient were put in contact (Table IV). Of interest is the loss of some activity by normal platelets when kept in contact with factor V-deficient plasma. This was interpreted as due to consumption of platelet factor V activity during contact, rather than to inhibition. Factor V-deficient platelets corrected a hemophilic mixture almost as well as normal platelets in a hemophilic system, demonstrating that the 2 activities are separable and that factors necessary for evolution of plasma thromboplastin *in vitro* may come interchangeably from platelets or plasma. If both plasma and platelets are deprived of the same factor, thromboplastin formation is defective. This was proved again by heating hemophilic plasma and thus destroying factor V activity; then attempting activation by factor V-deficient platelets. Under these conditions no source of factor V was present in the mixture with decreased thromboplastin

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Further Studies on Growth-promoting Factor of Exudates.* (25820)

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Earlier studies indicated that in inflammatory exudates there is present a diffusible growth-promoting factor capable of explaining reasonably the mechanism of repair following an inflammation(1,2). Repeated injections of this factor into breast tissue of non-pregnant rabbits induce lesions closely resembling chronic cystic mastitis(1,2). The cutaneous epithelial structures adjacent to treated nipples likewise display features of marked hyperplasia(2). Furthermore, evidence reported indicated that combination of growth factor of exudates and a carcinogenic hydrocarbon, methylcholanthrene, is followed by further enhancement of proliferative lesions(3,4). The concept has been developed that long standing inflammation may act as a cocarcinogen in presence of a carcinogen, be it exogenous or endogenous(4). The mechanism of action in this important role of inflammation in relation to development of neoplasia appears referable to liberation of growth-promoting factor of exudates(4). Evidence is here presented that activity of the factor appears destroyed by tryptic or ribonuclease digestion. Some of its biological properties are described.

Methods. The growth-promoting factor is present in concentrated diffusate of exudate and is obtained as described earlier(1,2). Chromatographic studies on acid hydrolyzate of the diffusate of exudates indicated association of a peptide with the active principle (2). Spectrophotometric absorption studies have also been conducted with a DU Beckman apparatus. With progress of inflammatory reaction from alkalinity to acidity(5), the diffusate assumes approximately the same pH as the exudate from which it has been obtained. The concentrated diffusate of alkaline exudates is adjusted with N acetic acid to approximately pH 4.5. A small precipitate forms, which augments further after refrigeration. It is then collected by centrifugation at 21,600 x g. This precipitate, washed twice with distilled water, is repeatedly injected into nipples of non-pregnant rabbits by technic previously described(1,2). Untreated nipples as well as nipples repeatedly injected with a buffer at pH 6.2 were likewise studied; the latter to control for the active growth factor, when injected in a medium at acid pH. Irreversibility of effects induced by growth-promoting factor has been tested by periodic observations of nipples for months following cessation of administration of the active principle. Chromatographic studies have been undertaken for presence of nucleotide structures in growth-promoting factor. The material is first hydrolyzed with 70% perchloric acid(6). Descending chromatography is uti-

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