quite young(8). An estrogenic response might be expected from the structural similarity of these compounds with the stilbestrols.

The reduction in thyroid weight seen with SC-3402 is unusual and may be related to the large thyroid size of the control animals in that particular lot. Likewise, the decreased ovarian and uterine weight in the rats on the lower dose of sulfoxone was not sustained with larger doses and is probably of no biological significance.

Summary. A sulfone, sulfoxone, and 2 nitrile steroid analogues, SC-3402 and SC-4473, have been tested in female rats for endocrine effects and compared with amphenone for reference. None of the 3 substances caused adrenal enlargement similar to that seen in the amphenone-treated animals. SC-4473 was markedly estrogenic. This substance represents the first nitrile to be shown to possess estrogenic activity in the rat.

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Serum Thrombotic Accelerator (STA) Activity: Its Relation to the First Phase of Clotting.* (26133)

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Normal mammalian serum, in striking contrast to plasma, induces thrombosis in areas of vascular stasis essentially devoid of endothelial injury(1,2). The activity of the moiety in serum responsible for this phenomenon (serum thrombotic accelerator, or STA activity) is mediated through the intrinsic clotting system and is demonstrable only in serum prepared from blood initially rich in Hageman factor (HF), plasma thromboplastin antecedent (PTA), and Factor IX (PTC)(3). The present report further characterizes the relationship between STA activity and these factors.†

Methods and materials. STA activity of human and hen serum was measured in the rabbit by a previously described assay using a standard dose of 1.32 ml/kg(5). When STA activity was measured in the hen using hen and normal human serum, doses as high as 2.64 ml/kg were employed. The amount of thrombus formed was scored on a scale from 0 to 4; a score of 0 represented no clot; a score of 1, a few macroscopic strands of fibrin; a score of 2, several small thrombi; a score of 3, two or more large thrombi; and a score of 4, or complete thrombosis, represented a single thrombus forming a cast of the isolated segment(5).

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† Presented in part before Am. Physiol. Soc. April 12, 1960, Chicago, Ill.(4).

Serum preparations. Serum was prepared from blood collected by direct venipuncture from normal subjects with uncoated needles and syringes. The blood was allowed to clot in glass tubes for 2 hours at room temperature, and was then left for 18 hours at 4°C. The serum was separated by centrifugation at 2500 g at 4°C for 20 minutes and stored in glass containers at –20°C. Such serum is hereinafter referred to as "contact" serum. Serum was prepared as described for "contact" serum except that collection, preparation, storage, and infusion were carried out with silicone-coated equipment.‡ Such serum is hereinafter referred to as "intact" serum. The effect of surface activation of STA activity was investigated with 3 additional serum preparations. Twelve silicone-coated glass beads (0.5 cm in diameter) were added to 10 ml samples of blood collected with siliconized equipment and gently mixed by inversion in siliconized test tubes until a solid clot had formed. The clotted blood was thereafter allowed to stand for 2 hours at room temperature and handled as previously described. An additional series of blood samples was similarly processed except that 12 uncoated glass beads were substituted for the coated beads. Finally, serum prepared with silicone equipment was stored in uncoated glass tubes at –20°C for 24 hours. All 3 serum preparations were tested for STA activity by infusion into rabbits.

Aliquots of "contact" serum maintained at 20°C, 37°C, and 56°C for 15 or 20 minutes were also infused into rabbits. "Contact" serum was adsorbed with celite§ (30 mg/ml) for 20 minutes and centrifuged at 2500 g and 4°C for 20 minutes to remove the celite powder. "Contact" serum adsorbed with chemically pure barium sulfate‖ (100 mg/ml) for 20 minutes was similarly prepared. The barium sulfate adsorbate was recovered by elution with sodium citrate as previously described. All 3 serum fractions were infused into rabbits at the standard dose(5). Decalcified human serum was prepared by addition of 1 volume of 0.1 M sodium oxalate, 1 volume of 0.1 M sodium citrate, or 1 volume of 0.29 M of disodium ethylenediaminetetraacetic acid (EDTA) to 9 volumes of contact serum. Hen serum was prepared from blood obtained by cardiac puncture according to the procedure used for "contact" human serum. Platelet-poor plasma(6) was infused into rabbits at the standard dose (1.32 ml/kg). Platelet-poor plasma, recalcified with equal parts of 0.025 M calcium chloride, was also infused into rabbits at a dose of 2.64 ml/kg after the fibrin clot had been expressed with a wooden applicator.

TABLE I. Effect of Temperature, Adsorption, Calcium, and Platelets on Human STA Activity.

Serum treatment	No. of rabbit infusions	Complete thrombosis (% animals)
Temperature ("contact" serum)		
20°C/20 min.	7	100
37°C/20 "	7	100
56°C/15 "	5	0
Fractionation ("contact" serum)		
Celite adsorption	4	100
$BaSO_4$ "	6	0
Sodium citrate eluate after $BaSO_4$ adsorption	6	100
Decalcification ("contact" serum)		
.1 M sodium citrate	2	100
.1 M " oxalate	2	100
.29 M EDTA	2	100
Plasma		
Platelet-rich (>300,000 cells/mm^3)	6	0
Platelet-poor (<100 cells/mm^3)	3	0
Recalcified platelet-poor plasma	3	100

Results. Table I shows assays of STA activity of human serum subjected to various physical and chemical modifications. Serum maintained at 20°C or at 37°C for 20 minutes contains full STA activity, whereas similar aliquots heated to 56°C for 15 minutes, lose this activity. "Celite" adsorbed serum exhibits complete STA activity in contrast to barium sulfate adsorbed serum. Two infusions each of serum decalcified with sodium oxalate, sodium citrate,

‡ Siliclad, Clay-Adams, Inc., New York; Monocote-E, Armour Lab., Kankakee, Ill.

§ Johns Manville, Inc., Boston, Mass.

‖ Bakers, Phillipsburg, N. J.

TABLE II. Relative Roles of HF, PTA, and Factor IX in Donor Serum and Recipient Animal.

Donor	Recipient	No. of infusions	Complete thrombosis (% animals)
Hen	Hen	8	0
"	Rabbit	6	0
Human	Hen	11	64
"	Rabbit	6	100

and EDTA were all capable of inducing thrombosis in rabbits. Finally, platelet-poor plasma lacks STA activity, whereas when recalcified this activity is demonstrable.

Hen serum is incapable of inducing thrombosis in hens or rabbits, whereas human sera can initiate intravascular coagulation in the hen as well as the rabbit (Table II). Of 11 human samples infused into the hen, 7 infusions yielded a score 4 thrombus; 2, a score 3 thrombus; 1, a score 2 thrombus; and 1 a score 0 thrombus. Thus, more than 90% of these infusions of human serum into hens demonstrated partial or full STA activity.

The effects of "surface" on STA activity of normal human serum are shown in Table III. STA activity is not demonstrable in "intact" serum in contrast to "contact" serum. It is evident from the 38 serum samples tested in the rabbit assay that activation of STA is dependent on exposure of "intact" serum to "foreign" surface.

Discussion. Previous studies have demonstrated that Factors I (fibrinogen), II (prothrombin), III (thromboplastin), V (ac-globulin), VII (convertin), VIII (antihemophilic factor), and X (Stuart factor) are not required in the donor serum for STA activity to be manifest(1,3,4). Results of infusions with recalcified platelet-poor plasma indicate that platelets, too, are not required for the elaboration of STA activity (Table I). Whereas HF, PTA, Factor IX, and possibly Factor IV (Ca^{++}) are essential for STA activation, the data in Table I indicate that STA is distinct from HF, PTA, and Ca^{++}. HF and PTA activities are relatively stable in serum at temperatures as high as 60°C(7,8). In contrast, STA activity is lost under these conditions. Moreover, barium sulfate adsorbed serum, rich in HF and PTA(7,9) cannot induce a thrombus, whereas the sodium citrate eluate of the barium sulfate adsorbate, relatively devoid of these 2 factors, can cause thrombus formation. In addition, "celite" adsorbed serum, also devoid of HF and PTA (10) retains the capacity to induce thrombi.

The hen, congenitally lacking in HF, PTA, and Factor IX(11) provides an opportunity to determine the relative roles of these 3 factors in donor serum and recipient animal. The data in Table II are consistent with previous observations(3) that these 3 coagulation factors are required for activation of STA in the donor serum and it is also clear that HF, PTA, and Factor IX need not be present, even in an inactive form, in the recipient animal for thrombus formation to occur.

Although STA activity can be distinguished from HF and PTA, no fractions in normal human serum have yet been prepared in which STA activity has been obtained free of Factor IX. The 2 serum fractions (Table I) exhibiting STA activity also contain Factor IX. Barium sulfate adsorbed serum, devoid of STA activity, lacks Factor IX. Serum prepared from patients

TABLE III. Effect of Surface on STA Activity of Human Serum.

Collection	Preparation	Storage	Infusion	No. of infusions	Complete thrombosis (% animals)
Glass*	Glass	Glass	Glass	6	100
Silicone	Silicone	"	Silicone	8	62
"	Silicone and glass beads	Silicone	"	6	60
"	Silicone and siliconized beads	"	"	9	11
" †	Silicone	"	"	9	0

* Contact serum. † Intact serum.

congenitally deficient in Factor IX exhibit no STA activity despite normal amounts of HF and PTA. This might suggest that the 2 entities, STA and Factor IX, are identical. However, in a study of the influence of Dicumarol on STA activity in dogs, it was shown that loss of this thrombotic activity was dissociated from the depression of any coagulation factor known to be reduced by Dicumarol singly, or in combination(12). Although a temporal correlation between loss of STA activity and depression of Factor IX occurred, a clear-cut dissociation between STA activity and Factor IX was, nevertheless, observed(12). Similar studies, currently in progress in man, show comparable results and indicate that STA and Factor IX may not be identical.

Even though STA activity is distinct from HF, the experiments with various types of "contact" and "intact" serum clearly indicate that elaboration of STA activity is dependent, in part, on prior activation of HF. Failure to achieve complete activation with uncoated glass beads may be the result of inadequate glass surface area. Similarly, the inability to prevent partial activation with siliconized glass beads may be attributed to incomplete silicone coating of the beads. These results may reflect the effect of exposed, uncoated surface on activation of HF.

Several investigators, using *in vitro* technics, have concluded that HF and PTA are the initial reactants in the sequential steps whereby intrinsic thromboplastin is elaborated(13,14,15), and that the "complex" formed by these 2 factors activates Factor IX before reacting with platelets or with any other coagulation factor involved in the first stage of clotting with the possible exception of Ca^{++}(15). Whether Ca^{++} is required in either or both of these steps *in vitro* has not yet been established(16). The data obtained in our laboratory, using *in vivo* thrombus formation as the endpoint, are consistent with these interpretations of *in vitro* observations. The findings with various types of "contact" and "intact" human serum emphasize the pivotal role of "surface" and HF activation in the elaboration of STA activity. It has also been observed in *in vitro* experiments that activation of HF can result in further activation of the components of intrinsic thromboplastin in absence of PTA. In contrast, no activation can be demonstrated when HF is absent(14). This finding provides a possible explanation for our earlier results wherein HF-deficient serum was essentially devoid of STA activity but PTA-deficient serum exhibited a significant though less than normal amount of STA activity(3).

From the viewpoint of the pathogenesis of thrombosis, STA represents a potent thrombotic activity, appearing early in the elaboration of intrinsic thromboplastin, distinct from platelets, tissue thromboplastin and thrombin and inducing a thrombus that initially is histologically dissimilar from that produced by tissue injury(2). The relevance of these observations to initiation of spontaneous intravascular thrombosis will depend, in part, upon elucidation of the role of endothelial surface in the activation of Hageman factor.

Summary. Serum thrombotic accelerator (STA) activity requires for its elaboration activation of HF, PTA, and Factor IX, but does not depend on platelets or any other recognized coagulation factor with the possible exception of Ca^{++}. Although essential in the donor serum for formation of this thrombotic moiety, HF, PTA, and Factor IX need not be present in the recipient animal for thrombus formation to occur. STA activity is distinct from Ca^{++}, HF, and PTA, and may not be identical with Factor IX; however, activation of HF appears to be the initial reaction required in the elaboration of this potent thrombotic agent that is distinct from thrombin and tissue thromboplastin.

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