thereby increasing circulating nutrients and hormones available to the mammary gland.

Relaxin rather than P acts upon the anterior pituitary to stimulate increased secretion of mammogen. This theory would assign P to an indirect role in mammary gland growth although it is possible that P and relaxin are equally effective at the pituitary level.

The hormones and glands involved in endogenous relaxin secretion become of paramount importance if relaxin rather than P stimulates pituitary mammogen secretion and lobule alveolar growth. Since the growth of the mammary gland of male animals is stimulated by E and P, it would follow that relaxin may be secreted in the male although, to date, relaxin has not been detected in male animal blood following injection of E and P.

Summary. 1) Using DNA as index of mammary gland lobule-alveolar growth in intact and castrate male mice, it was shown that daily injection of 0.75 μg estradiol benzoate and 2.5 GPU of relaxin for 10 days stimulated increases in DNA equal to that produced by the same level of estrogen and 0.75 mg progesterone. Therefore, relaxin no longer need be considered merely a synergist of estrogen and progesterone in mammary gland growth. 2) Estrogen and relaxin at above levels were without effect upon mammary alveolar growth in hypophysectomized mice. Relaxin, therefore, is not comparable to pituitary mammogen as a stimulator of mammary gland growth. 3) It is suggested estrogen and progesterone stimulate endogenous secretion of relaxin and relaxin, in turn, stimulates increased secretion of mammogen by the pituitary.

1. Kirkham, W. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 139.
2. Smith, T. C., *Endocrinol.*, 1955, v57, 33.
3. Turner, C. W., Gomez, E. T., *Mo. Agr. Exp. Sta. Res. Bull.*, 1934, #206.
4. ———, *ibid.*, 1934, #207.
5. Yamamoto, H., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 130.
6. Yamada, J., Nagai, J., Naito, M., *Endocrinol. Japonica*, 1954, v1, 63.
7. Hamolsky, M., Sparrow, R. C., PROC. SOC. EXP. BIOL. AND MED., 1945, v60, 8.
8. Smith, T. C., *Endocrinology*, 1954, v54, 59.
9. Garrett, F. A., Talmage, R. V., *J. Endocrin.*, 1952, v8, 336.
10. Trentin, J. J., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 9.
11. Elliott, J. R., Turner, C. W., *ibid.*, 1951, v77, 320.
12. ———, *ibid.*, 1953, v82, 234.
13. ———, *Mo. Agr. Exp. Sta. Res. Bull.*, 1953, #537.
14. Kirkham, W. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1954, v83, 123.
15. Damm, H. C., Turner, C. W., *ibid.*, 1957, v95, 466; 1958, v98, 192.
16. Webb, J. M., Levy, H. B., *J. Biol. Chem.*, 1955, v213, 107.
17. Turner, C. W., Gomez, E. T., *Mo. Agr. Exp. Sta. Res. Bull.*, 1933, #182.
18. Mixner, J. P., Turner, C. W., *ibid.*, 1943, #378.
19. ———, *Endocrinol.*, 1942, v30, 591.

Received June 5, 1958. P.S.E.B.M., 1958, v99.

Surface Activation of Plasma Clotting: a Function of Hageman Factor.* (24294)

CHARLES L. JOHNSTON, JR., JOHN H. FERGUSON AND FRIEDA A. O'HANLON
Department of Physiology, University of North Carolina, Chapel Hill

The influence of surface contact on blood clotting has been recognized since the observations of Hewson (1770), Lister (1863), and Freund (1886). Modern literature contains many experiments which have attempted to implicate one or other of the clotting factors. Previously, no one explanation of the effect seemed adequate. Recent evidence suggests that Hageman factor may have a role in surface phenomena(1,2,3). In this communica-

* This investigation was supported by research grant from U.S.P.H.S., N.I.H.

tion, experimental data are presented on 4 new cases of Hageman trait and other factor deficient plasmas, their reaction on exposure to asbestos surface, and a comparison of the effect of addition of 'purified' Hageman factor to normal and deficient plasmas. The results point to a key role of the Hageman factor in surface effects on blood clotting and show that other clotting factors are not involved.

Materials and methods. Normal (N) plasmas were used as fresh, *aged* (AcG-, *i.e.* proaccelerin poor), or $BaSO_4$ fractions ('supernate' and 'eluate'). Preserved (lyophilized or frozen at –20°C) plasmas from patients with clotting factor deficiencies were obtained from our diagnostic laboratory, directed by Dr. John B. Graham, operated jointly by Pathology and Physiology departments. These comprised a test panel of plasmas and included Hageman deficient (Hag.-), hemophilic (AHF-), Christmas disease (PTC-), hypoproconvertinemic (Proc.-), and Stuart deficient (Stuart-). The diagnosis of Hageman trait was confirmed by cross match testing of our first patient's (L.C.) plasma with that of the original Hageman, provided by Dr. O. D. Ratnoff. Ratnoff's technics (3, personal communication) were followed in efforts to prepare 'purified' Hageman factor from sera of (A) recalcified, normal, outdated bank blood, (N) fresh normal, and (H and I) 2 Hageman trait cases, A.M. and J.I.† Asbestos powder was prepared and used as follows: asbestos (Baker's medium-fiber, acid washed) was powdered under water in a Waring Blendor, water removed by suction filtration, and residue dried at 200°F. After drying, clumps were reduced with mortar and pestle. The product was a reasonably uniform, grayish-white powder. Thirty mg were added to 1 ml of various plasmas, and agitated at intervals for 30 minutes at room temperature. The supernate, recovered by centrifugation, was designated *treated* or *activated*. Preliminary tests showed no further effects on increasing either amount of asbestos or contact time. Several *clotting tests,* some with modification, were used to see if, independent of their original purpose, they could serve the needs of our inquiry. These tests, one-stage systems, timed clotting at 37°C on addition of a thromboplastic agent and calcium to test plasma (or mixture). The prothrombin time (PT) test using 0.1 ml plasma, 0.1 ml thromboplastin and 0.1 ml 0.02 M $CaCl_2$ was the prototype. Plasma mixtures with appropriate factor-free substrates were used in specific assays(4) for prothrombin, proaccelerin, or proconvertin and/or Stuart factor. We used a saline extract of acetone dried human brain powder as thromboplastin. When cephalin, obtained by chloroform extraction of the human brain powder, was substituted for thromboplastin, the PT was converted into the partial thromboplastin time test (PTT)(5) and the standard proconvertin assay into a 'modified' proconvertin assay. The Stypven time used Russell's viper venom as the thromboplastic agent. In the new uses, the test system terminology was for identification and no longer had the original significance.

TABLE I. Asbestos Treatment—Normal Plasma. 7 test systems. Clotting-times, sec., 37°C.

Test system	Untreated	Treated
Partial thromboplastin time (PTT)	75.5	60
Prothrombin time (PT)	11.7	10.7
Standard proconvertin	18.5	13.8
Modified "	107.0	66.4
Stypven time	16.8	17.3
Specific one-stage prothrombin	22.9	22.7
" " proaccelerin	25.0	26.3

Results. Asbestos activation. Table I shows representative results obtained when normal human plasma was exposed to powdered asbestos. Clotting-times shorter than controls were obtained in 4 of 7 test systems. No significant alteration was noted in 3 assays. Similar results were obtained with bovine plasma and oxalate or citrate as anticoagulant. *Shortening of test clotting-times will be referred to as plasma activation by surface action of asbestos.*

The plasma panel was tested for asbestos activation by the standard and modified proconvertin assays. Table II shows that no significant change in lyophilized Hag.-

† Blood from this patient was made available through the courtesy of Dr. A. I. Chernoff, Vet. Adm. Hosp., Durham, N. C.

TABLE II. Asbestos Treatment—Various Plasmas. Clotting-times, sec., 37°C, in 2 types of test, (a) before and (b) after asbestos treatment of plasmas.

Plasma tested	Standard proconvertin test (a) Untreated	Standard proconvertin test (b) Treated	Modified proconvertin test (a) Untreated	Modified proconvertin test (b) Treated
Normal	18.5	13.8	107.0	66.4
Hag.-	*24.4*	*24.2*	*135.7*	*135.5*
AHF-	16.6	14.9	108.4	85.4
PTC-	22.0	19.4	98.8	70.9
AcG-	16.6	15.4	101.0	87.4
Stuart-	97.9	73.9	183.0	143.5
Proc.-	113.9	88.6	130.0	92.1

plasma resulted from such treatment. However, all other plasmas showed some degree of shortening (acceleration) of post-treatment clotting-times.

The response of normal $BaSO_4$ fractions to asbestos was studied. These fractions and Hag.- were treated separately, then mixed, assayed by the 2 proconvertin tests, and compared with untreated mixtures. The eluate was not activated when tested either alone or mixed with Hag.-. $BaSO_4$ treatment removed proconvertin and Stuart factor to which these assays were sensitive; hence, the supernate alone did not give measurable clotting-times. However, the mixture of treated supernate and Hag.- gave times *shorter* than the untreated control. This shortening resulted from treatment of the supernate, since Hag.- alone (Table II) was not activated. These data indicated that some plasma factor, present in normal plasma, not adsorbed by $BaSO_4$, and lacking in Hag.- was responsible for surface activation. We have been unable to confirm that a "small amount" of Hageman factor may be adsorbed(3).

Table III shows PTT data for control substrate clotting-times and equal volume mixtures of substrate and untreated test plasmas. Asbestos treated normal plasma shortened clotting-times more than untreated normal when mixed with *all* substrate plasmas. Treated Hag.- did not additionally shorten *any* substrate clotting-time below pretreatment levels. Indeed, in some instances there was lengthening instead of shortening; this is being investigated. Minor secondary clotting factor deficiencies are represented in Table III by failure of untreated normal to correct completely deficiencies in substrate plasmas. These do not preclude reaching the significant conclusion that Hageman factor deficient plasma fails to be activated by asbestos.

To show that the noted phenomena were unrelated to storage and manipulation of panel plasmas, fresh normal, AHF-, and Hag.- plasmas were obtained and tested immediately. Prothrombin time (PT) and partial thromboplastin time (PTT) test were used. There was failure of Hag.- to be activated: PT 12.1″ before, 12.2″ after treatment and PTT 286″ before, 319″ after treatment. The other 2 samples showed activation: AHF-: PT 12.3″ before, 10.6″ after treatment and PTT 216″ before, 157″ after treatment; normal: PT 12.4″ before, 8.8″ after treatment and PTT 92″ before, 60″ after treatment. Thus, freshly obtained materials reacted to asbestos in the same manner as the stored, and the noted minor secondary deficiencies were not part of the phenomenon. Since PT data on stored plasmas were of questionable significance, these fresh plasmas gave the best evidence that surface activation influenced the PT test. Only minor differences were noted when silicone was used to minimize initial surface effects.

Effects of purified Hageman factor prepa-

TABLE III. Effect of Asbestos Treatment on Normal and Hageman Deficient Plasmas: PTT Controls for Substrate Plasmas, and Mixtures of Equal Parts Test and Substrate Plasmas, before and after Treatment. Clotting-times, sec., 37°C.

Test plasma	Substrate plasma: Normal	AHF-	PTC-	Hag.-	Stuart-	Proc.-	AcG-	$BaSO_4$ supernate
Substrate, untreated	75.5	212	488	860	291	115.5	442	
Normal, "	75.5	80.2	172	118.2	107.6	124.2	87.2	91.4
Normal, treated	60.0	74.2	102	100	90.8	90.6	70.0	73.0
Hag.-, untreated	122.4	89.6	212	860	210.8	157.0	136.7	140.7
Hag.-, treated	125.0	91.5	434	825	241.6	172	140.8	150.0

TABLE IV. Tests of "Purified" Hageman Factor Preparation (N) and (H): Results of Addition to Normal and Hag.- Plasmas. Clotting-times, sec., 37°C.

Test material .09 cc	.01 cc	PTT
Oxalated normal	+ Barb.-sal. buffer	79.8
	N (1:1)	39.0
	H (")	84.3
	N (1:10)	60.4
	H (")	91.8
Hag.- (L.C.)	+ Barb.-sal. buffer	402
	Oxalated normal	140.0
	N (1:1)	91.4
	H (")	313

rations. Addition, in 10% volume, of varying dilutions of purified preparation (A) to normal plasma resulted in PTT shortening, which was marked on addition of undiluted (1:1) material, and demonstrable at 1:100 but not 1:200 dilution. The normal plasma presumably had adequate Hageman factor, so that these results indicated: a) a highly potent concentrate, and b) no evidence of optimum for Hageman factor.

Table IV compared addition of purified preparations (N) and (H) to normal and Hag.- plasmas. (N) markedly accelerated both. However, (H) was ineffective when added to normal and had only minimal effect on the Hag.-, despite the fact that the anticipated concentration of *any* Hageman factor present should have been 100x (see above). When compared with addition of an equivalent volume of normal plasma, (H) contained only a trace of Hageman factor. Similar data were obtained with (I).

Table V gives additional evidence that (H) was inactive, since it failed to shorten substrate plasma clotting-times. Comparably, (N) shortened clotting-times of all substrate plasmas. Another important conclusion is that purified preparations did not contain factors other than the Hageman factor, since no shortening was noted in *any* of the deficient substrates. Thus, similarity of action of the purified preparation to asbestos activated plasma (Table III, V), must be due to the presence or absence of Hageman factor.

Discussion. Asbestos treatment produced "activation" (clotting-times shorter than controls in several test systems) in all plasmas or fractions tested except those lacking Hageman factor. Since 'purified' Hageman factor preparations from normals, *but not Hageman trait patients,* mimicked this clotting-time shortening, it is concluded that Hageman factor is responsible for the phenomenon.

These data permit a new look at previously published work suggesting that other clotting factors are responsible for surface activation of plasma. Alexander(6) summarized the platelet role . . . "Precisely which clotting factor (or factors) is directly influenced by surface is far from clear. Contrary to earlier opinion, it appears that it is not the platelets." Indeed our earlier work showed surface effects in completely platelet-free plasma (7). Those clotting factors most usually held responsible for surface activation of plasma were PTC and proconvertin. Our data showed surface activity in plasma lacking these factors, but containing Hageman factor. Similarly, others(1,2) showed that plasmas lacking either or both PTC and proconvertin had surface activity. Shafrir and de Vries(2) showed activity in plasma devoid of AHF, PTC, prothrombin, proconvertin, and fibrinogen. We confirmed and extended this

TABLE V. Tests of "Purified" Hageman Factor Preparations (N) and (H): Partial Thromboplastin Testing of .01 cc Test Material and .09 cc Plasma Substrate. Clotting times, sec., 37°C.

Test material	Substrate: Normal	AHF-	PTC-	Hag.-	Stuart-	Proc.-
Barb.-sal. buffer	85.2	156.0	253	234.0	342.6	129.0
Normal plasma	82.2	102.8	159	178.6	159.2	115.4
N (1:10)	69.1	135.4	234	131.4	307.0	125.4
H (")	81.2	156.0	276	253.3	334.2	132.7
N (1:1)	44.2	91.8	179	75.8	182.0	79.6
H (")	81.1					131.8

latter finding and showed in addition that, as implied by Ratnoff and Margolius(3), surface activation is activation of Hageman factor. Finally, by use of purified Hageman factor devoid of other clotting factors, we mimicked the clot acceleratory activity of asbestos treatment. Comparable preparations from Hageman trait patients were inactive.

Removal of an inhibitor must be considered as an alternative explanation of our data. Shafrir and de Vries(2) believed that an inhibitor was not adsorbed onto glass. We did not feel that asbestos removed an inhibitor, since: 1) treated Hageman deficient plasma did not shorten clotting-times of plasmas containing Hageman factor (Table III); 2) asbestos treatment was mimicked by positive factor addition (Tables III, V); 3) there may have been *increased* inhibition which resulted from asbestos treatment (Table III).

Thus, surface contact may be said to result in Hageman factor activation. In its absence, no activation is noted. The failure of asbestos to activate plasma lacking Hageman factor suggests a *presumptive* test for Hageman trait diagnosis.

Summary. 1. Asbestos treatment of plasma produced activation (clotting-time acceleration) in normal and AHF, PTC, proconvertin, prothrombin, proaccelerin and Stuart deficient plasmas. Hageman deficient plasma was not activated by comparable treatment. 2. Purified Hageman factor preparations from normal sera simulated activation, whereas preparations from sera of Hageman trait patients were inactive in the same test system. 3. The phenomenon of surface activation resulted from Hageman factor activation and was not due to inhibitor adsorption. 4. Asbestos treatment of plasma may be used as a *presumptive* test for Hageman trait.

1. Margolis, J., *J. Physiol.*, 1957, v137, 95.

2. Shafrir, E., de Vries, A., *J. Clin. Invest.*, 1956, v35, 1183.

3. Ratnoff, O. D., Margolius, A., Jr., *Hemophilia and Hemophilioid Diseases,* K. M. Brinkhcus, ed., 1957, 124.

4. Lewis, J. H., Ferguson, J. H., Fresh, J. W., Zucker, M. B., *J. Lab. Clin. Med.*, 1957, v49, 211.

5. Rodman, N. F., Jr., Barrow, E. M., Graham, J. B., *Am. J. Clin. Path.*, 1958, v29, 525.

6. Alexander, Benjamin, *N. Eng. J. Med.*, 1955, v252, 432.

7. Ferguson, J. H., *Blood Cells and Plasma Proteins,* J. L. Tullis, ed., 1953, 93.

Received June 11, 1958. P.S.E.B.M., 1958, v99.

Metabolism of Sr^{90} in Adult Beagle Dogs.* (24295)

BETSY J. STOVER AND DAVID R. ATHERTON (Introduced by T. F. Dougherty)
Radiobiology Laboratory, University of Utah, Salt Lake City

This study of Sr^{90} metabolism is part of a large program to compare the chronic toxicities of Ra^{226}, Pu^{239}, Ra^{228}(MsTh), Th^{228} (RdTh), and Sr^{90} in young adult beagle dogs. The experimental design, especially relationship of dose levels to maximum permissible and natural levels in man, has been described (1). The basic plan is to compare the effects of these radioactive isotopes in a larger, longer-lived animal (the dog) than is usually used in the laboratory; then to use this information in conjunction with the knowledge of radium poisoning in humans to reappraise maximum permissible levels for other isotopes. To correlate the biological effects with radiation dose, a detailed knowledge of the metabolism of each radioisotope is needed.

Methods. Sr^{90}, with its daughter Y^{90} in equilibrium, in a citrate buffer solution of pH = 3.5, was given in single intravenous injection. Injected doses ranged from 0.56 to 100 μc/kg; duplicate doses served as injection standards. The beagle dogs, healthy young adults 16 to 17 months old, during the excretion study were housed in stainless steel metabolism cages, with perforated floors which permit urine to drain into plastic bottles so that

* Supported by the U. S. Atomic Energy Comm.