Blood-Clotting Studies on Dogs Internally Irradiated with Radio-Gold.* (19684)

JOHN H. FERGUSON, G. A. ANDREWS, AND MARSHALL BRUCER.

From the Department of Physiology, University of North Carolina, and Oak Ridge Institute of Nuclear Studies.

Radio-gold (Au^{198}) was administered intravenously as colloidal sol to 5 adult dogs in single dose approximating 20, 10, 10, 5, and 1.6 millicuries per kg, respectively. Two dogs received stable colloidal gold in volumes, per kg of body weight, comparable to the 10 millicuries per kg radio-gold experiments. An untreated control animal was kept with the irradiated dogs in an isolation hut and at no time showed any abnormal findings. Prior to the injections all animals were apparently healthy and gave normal prothrombin consumption tests. The dogs were observed carefully throughout the experimental period and autopsy findings were supplemented by extensive histopathological studies, details of which will be reported elsewhere. During life they were subjected to repeated hematological observations, including routine platelet counts. Special blood-clotting studies were made as described, with particular emphasis upon "prothrombin consumption tests" by both the 2-stage and modified one-stage methods. Whole blood clotting-times at 37°C were noted in conjunction with these tests and incidental observations were made on clot retraction both in 1-1½ hours and after 24 hours. Fibrinolysis was also looked for at the end of 24 hours (at 37°C) but was invariably absent. Icteric sera were particularly noted and observed in the later stages in dogs receiving 10-20 millicuries per kg.

Methods. Prothrombin consumption tests, etc. Jugular vein blood was secured with needle and syringe, starting a timer at midpoint of bleeding. 2.7 ml blood was placed in each of 7 marked tubes. Tube 1, containing 0.3 ml of 0.1 M sodium oxalate, was shaken and immediately centrifuged (3 min. at 3000 r.p.m.) for plasma. The other tubes were placed in a water bath at 37°C and observed for *whole blood clotting-time* (c.t.). *Clot-retraction* was noted in remaining tubes at 1-1½ hours and the last tube reobserved at 24 hours. *Clot-lysis* was also looked for, but was invariably absent. Tubes 2-6 were oxalated, respectively, at 6, 15, 30, 60 + c.t., and 90 minutes after bleeding (timer zero), loosening the clot with a glass rod and centrifuging for serum. *Thrombin test.* 0.1 ml serum (or plasma) + 0.2 ml fibrinogen, at 37°C, showed no thrombin in the plasma and very little in any of the sera, clotting-times usually being over an hour. *One-stage (modified) prothrombin clotting-time (PCT) test* (1). Mixtures of 0.1 ml each of test serum (or plasma), $BaSO_4$-adsorbed normal dog plasma (to supply fibrinogen), thromboplastin (Schieffelin's "soluplastin"), and 0.04 M $CaCl_2$ were timed for clotting at 37°C. *Two-stage prothrombin assay.* The Iowa method, as modified by Ware and Seegers(2), was closely followed, with the very minor substitutions of (a) 10 unit/ml Upjohn's bovine thrombin, for defibrination (1st step), required for plasma and 6 minutes serum only; (b) choice of either 1) Difco incubation mix (courtesy Dr. C. W. Christensen) or 2) mixture of 10.6 ml saline (0.85% NaCl), one ml imidazole buffer (pH = 7.3), 4 ml 15% *purified* acacia, 0.6 ml thromboplastin (Schieffelin's "soluplastin"), 0.9 ml 0.1 M $CaCl_2$; (c) dog fibrinogen, prepared by adsorbing normal oxalated plasma with $BaSO_4$, precipitating with one-fourth saturated $(NH_4)_2SO_4$, and dialyzing against saline containing 1:200 of 38% trisodium citrate. Tests were made at 28°C and units per ml computed from total dilution multiplied by factor (from Seegers' table) to correct for clotting-times other than 15 seconds. *Test for circulating anticoagulant, etc.* Clotting-times, at 37°C,

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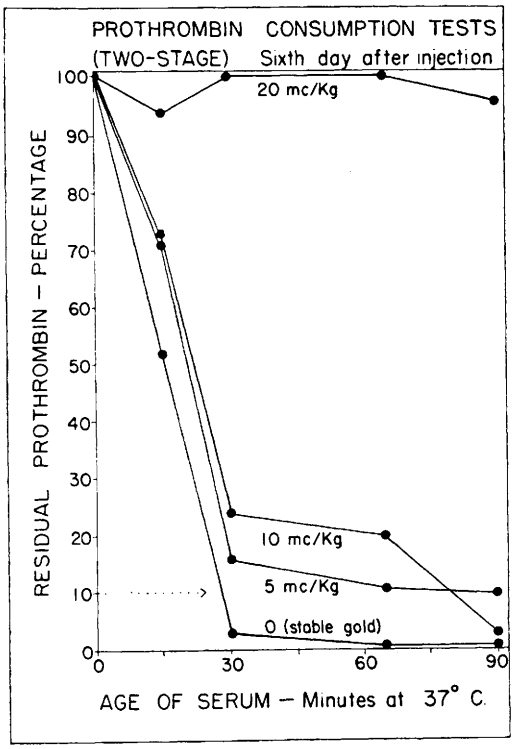

FIG. 1. Prothrombin consumption tests (2-stage). Sixth day after inj.

of mixtures of (a) normal dog plasma: 0.2, 0.15, 0.1, 0.05, 0.02, 0.01, 0; (b) test plasma: 0, 0.05, 0.1, 0.15, 0.18, 0.19, 0.2, respectively, recalcified with 0.2 ml 0.02 M $CaCl_2$. A heparin-antithrombin type of circulating anticoagulant would increase clotting-times at the start of the series, whereas serious clotting defects (*e.g.*, hemophilia, severe hypoprothrombinemia, or lack of accelerin or proconvertin) would give longer clotting-times in the later mixtures.

Prothrombin consumption tests: 2-stage method. Fig. 1 shows the results of tests made on the 6th day after the gold injection. The residual prothrombin, assayed by the "2-stage" method, is recorded, as percentage of the original (*zero* time) plasma unitage, for serum removed after oxalation of consecutive blood samples aged at 37°C, respectively, for 15, 30, 60 + c.t., and 90 minutes after withdrawal from the jugular vein. The control, injected with stable gold, is quite normal, with only negligible amounts of prothrombin remaining after 30 minutes. Anything more than 10% residual prothrombin in the 60-90 minute period is significant evidence of impaired prothrombin utilization. As may be seen, this occurred in the 3 irradiated animals, being extremely marked at the 20 millicuries dosage. These findings were confirmed, somewhat more strikingly, in data obtained subsequently, especially when the effects of the internal irradiation on the prothrombin utilization were maximal (Fig. 2).

Fig. 3 shows, for all test animals, the course of the phenomenon, as indicated in the data of the 90-minute tests of observations repeated at intervals throughout the study period. Significant observations are summarized as follows: A. The 2 animals receiving stable gold remained normal, showing mere traces of residual prothrombin, in observations over a 2-week period. Sacrificed 5 months later, their livers were normal. B. One animal received a token dose of 1.6 millicuries/kg of radiogold. Its tests remain below the significant 10% level. Sacrificed on the 106th day, its liver and other organs were normal.

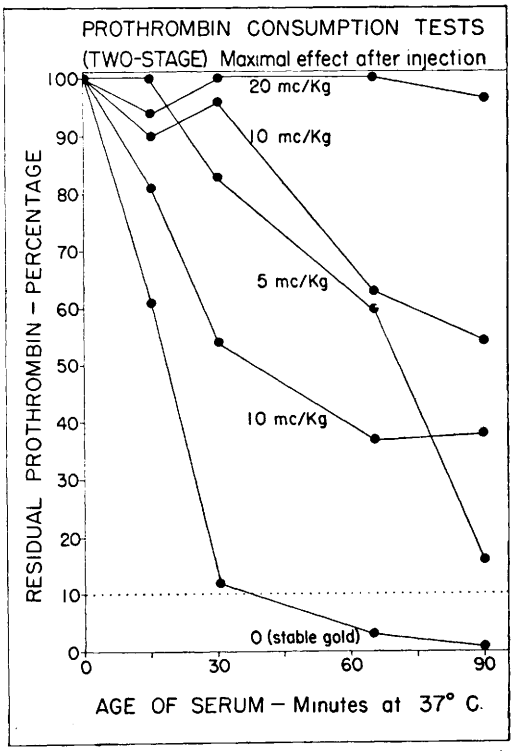

FIG. 2. Prothrombin consumption tests (2-stage). Max. effect after inj.

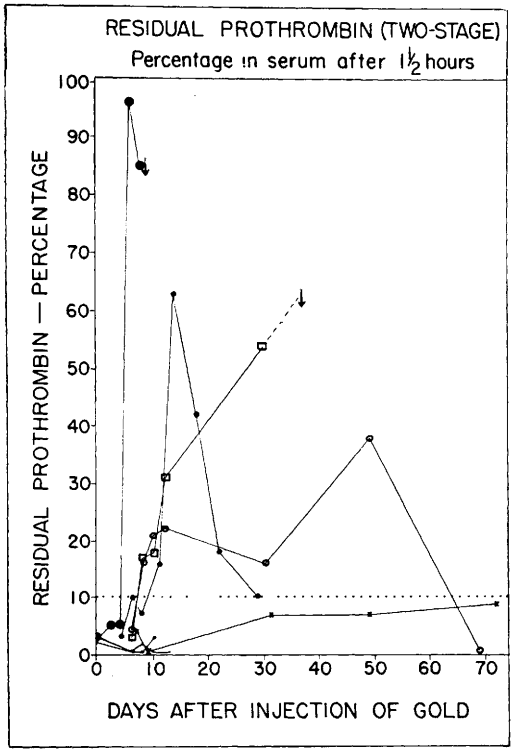

FIG. 3. Residual prothrombin (2-stage). % in serum after 1½ hr (see text). Arrow indicates death of dog.

C. The dog receiving the 5 millicuries dose showed positive tests on the 6th day, increasingly so until the 18th day (a severe reaction) and return toward normal by the 29th day. It is surviving, now 3 months later. D. In one animal receiving 10 millicuries per kg, the test became positive on the 6th day, more so through the 12th day, fluctuated in severity at the 30th and 49th day, and was back to normal on the 69th day. Marked jaundice was noted through the second month but lessened, and death on the 83rd day was unexpected. Autopsy, however, revealed marked liver damage reported as "chronic cirrhosis, with atrophy of liver cords, engorged sinusoids, and considerable hemosiderin, apparently from old hemorrhage." E. In the other animal receiving 10 millicuries radiogold, the course during the first 10 days was very similar to the foregoing. Thereafter, the prothrombin consumption test data, icterus, and clinical conditions were more severe and progressive and the animal died on the 37th day. In addition to widespread (particularly pulmonary) hemorrhages, typical of the radiation syndrome, the autopsy showed acute liver damage reported as "massive and complete hemorrhagic necrosis." F. In the last animal receiving 20 millicuries radiogold, the prothrombin tests (as in the other cases) were normal before the 6th day. There then occurred an almost complete failure of prothrombin utilization. Severe jaundice was noted at this time and death occurred on the 9th day, with typical radiation syndrome hemorrhages. At autopsy, the liver was markedly hemorrhagic, icteric, and necrotic. A section of duodenum, which nestled under the liver, was swollen by a massive interstitial hemorrhage. There was bleeding into the neck from the simple venipuncture of the previous day. The hemorrhages, stated to be "typical" of the radiation syndrome, as noted in the last 2 animals, included: bleeding into the lungs, petechiae in serous membranes (subendocardial, subepicardial, peritoneal), spotty hemorrhages in mucosa of the alimentary canal, lymph node hemorrhages, and (in the 10 millicuries animal) extensive bleeding into the mesentery and retroperitoneal tissues.

Tests by one-stage method. Fig. 4 summarizes the significant results of prothrombin consumption tests made by the modified *one-stage* method, on the same samples as used for the 2-stage tests. The chart records actual clotting-times, at 37°C, of mixtures of test serum (or plasma), $BaSO_4$-adsorbed normal dog plasma (to supply fibrinogen), thromboplastin, and calcium. The longer the clotting-time, the less the prothrombin. The dotted line, at a value of 10 sec., divides the data into 2 groups. Those that are "normal" in showing values above this level by the 30 min. test period (and thereafter) include: (a) all tests before the gold injections on the 5 test animals, (b) the 2 additional animals (A,B) receiving stable gold; and (c) the animal (X) after receiving only 1.6 millicures/kg of Au^{198}.

The "positive" group includes the 4 animals irradiated at 5-20 millicuries/kg. In their sera, the clotting test values remain well below the 10 sec. "critical level" in observations during the pathological period. Qualitatively, at least, this confirms the findings by the 2-

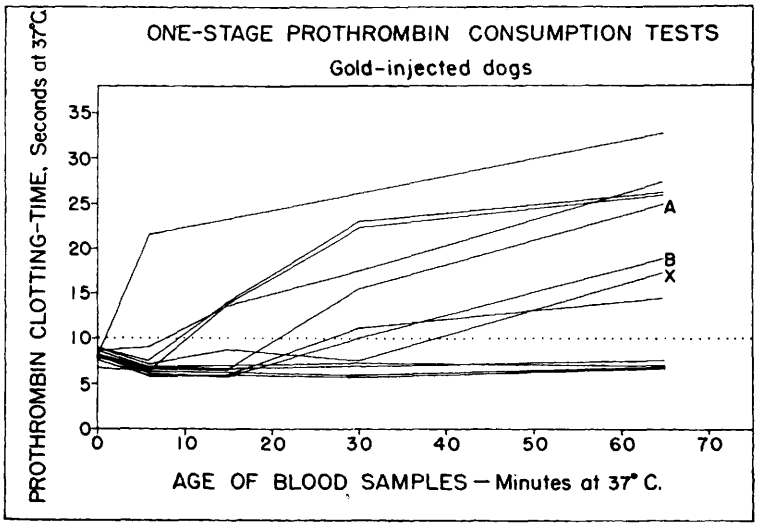

FIG. 4. One-stage prothrombin consumption tests. Gold-inj. dogs (see text).

stage method. Strict quantitation by the one-stage method, however, presents some difficulties. One of the reasons for this is suggested by a finding, as can be seen from the chart, that the PCT values in the earlier tests show a drop, instead of the increase seen later in the "normals." Without discussing this phenomenon, previously reported by others(3), we may state that it seems to be a normal result of the appearance, during blood clotting, of some accelerator-type factor which influences the one-stage test. We would agree that it might be Alexander's SPCA or Owren's convertin(4). It is significant that this appearance is evident in all the irradiated animals' sera, suggesting that they are normal in respect to this factor.

Tests for circulating anticoagulant, etc. On several occasions we tested for the possibility of a circulating anticoagulant by measuring recalcification clotting-times of mixtures of the test animal's plasma and normal plasma. As shown in Table I (first row) there is no significant c.t. difference and hence no evidence for circulating anticoagulant or other abnormality which these tests could have revealed. In fact, with the use of an older "normal" plasma in the second series of tests, the test plasma lessened clotting-times toward the usual value from which the particular normal dog plasma had deviated, owing, in all probability, to loss of accelerin or labile factor(4). Penick(5) has reported that the antihemophilic factor remains normal in X-irradiated dogs and his and Cronkite's radiation studies (4,6) cover many of the points we have mentioned.

TABLE I. Test for Circulating Anticoagulant, etc. Plasma-recalcification clotting-times, seconds at 37°C. Dog receiving 10 mc per kilo radio-gold (Au^{198}).

% test plasma	0	25	50	75	90	95	100
% normal "	100	75	50	25	10	5	0
10th day after inj.	55	56	57	56.3	61	59	63
12th " " "	123	73.5	62.5	63.8	60.3	62	59.3

Other data. In our internally irradiated animal plasmas, there was no hypoprothrombinemia. Whole blood clotting-times were all normal except for one test (on the 6th day) in the 20 millicuries/kg dog, when it was about twice normal. Clot-retraction was definitely lessened in the 6-8 day period on this 20 millicuries animal, and in the 10-30 and 10-40 day periods on the two 10 millicuries animals.

Platelets. Our platelet counts, made by a good hospital technician, while perhaps not up to the highest research standards, give acceptable data which are shown in Table II. There seems to be some lack of correlation with the results of the 2-stage prothrombin tests, particularly in the appearance of "positive" tests in 2 animals on the 6th day with

TABLE II. Comparison of Platelet Counts (A)* and Residual Prothrombin (B)† in Dogs Injected with Radio-Gold.

	Dosage of radio-gold					
	20 mc/kg		10 mc/kg		5 mc/kg	
Day after inj.	(A) × 100	(B) %	(A) × 100	(B) %	(A) × 100	(B) %
0	3545	4	2384	—	3125	—
2	2175	7	7650	—	—	—
4	2425	5	—	—	3375	5
6	450	100	3396	20	3725	11
8	225	86	1394	41	1350	3
10	(Died 9th day)		2190	27	—	60
13			260	88	775	67
18			131	63	1450	34

* (A) Platelet counts: per mm³.
† (B) Residual prothrombin: % left in 60 min + clotting-time, by 2-stage test.

normal platelet levels and divergent results on the 8th day, when both animals showed about the same degree of thrombocytopenia. In later tests the severe thrombocytopenia and markedly "positive" prothrombin consumption tests do go together. We accord much respect to the cited data of Cronkite and of Penick on this topic. It is certainly true, and we have data from our own laboratory to prove it, that platelets are essential for plasma clotting, normally. They do act, however, in conjunction with the plasma factors and I believe we need to investigate the latter more fully before concluding that the defect of prothrombin consumption in radiation sickness (resembling that seen in thrombocytopenia(7) and in hemophilia(3)) is solely due to reduction in the number or quality of the blood platelets.

Summary. Internal irradiation with radio-gold (Au^{198}), in sufficient dosage, and after several days' latency, induces a blood coagulation defect of which the prothrombin consumption test is a sensitive index. By using this test through the course of the irradiation, the 2-stage values seem to reflect, with some accuracy, the severity and probable outcome of the radiation injury. Similar data are becoming available in several animal species (including man) and with various types of irradiation. We have begun to collect data on human cancer patients under isotope radiation therapy in order to learn more about this interesting phenomenon.

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