Whole Blood Clotting, Clot Retraction and Prothrombin Utilization in Burros Following Total Body Gamma Radiation.* (20113)

BERNARD F. TRUM† AND JOHN H. RUST.† (Introduced by John H. Ferguson.)

From the University of Tennessee, Atomic Energy Commission Agricultural Research Program, Oak Ridge.

The total-body gamma radiation syndrome as seen in the burro is similar to that reviewed by Cronkite and Brecher(1) for common laboratory animals and man. The hemorrhagic phase consisted of purpura, petechial hemorrhage and pancytopenia of blood and bone marrow elements(2).

In a group of burros subjected to lethal amounts of gamma radiation a particular study was made of the clotting mechanisms, especially whole blood clotting, clot retraction and prothrombin utilization. The results of that investigation are herewith reported.

Materials and methods. Nine healthy female burros, 3 to 4 years old, were exposed to 950 r of gamma radiation, which was about 90% lethal within 30 days for burros ($LD_{90/30}$). The exposure device has been described by Wilding *et al.*(3) and the dosimetry calculated in a manner described by Rust *et al.*(4). Pre-irradiation values established each animal as its own control. Methods were controlled by concurrent tests on non-irradiated animals. Blood was collected by jugular venipuncture directly from 15 ga. needles into 4 calibrated 13 × 100 mm pyrex tubes. Exactly 4.5 ml of blood was placed in each tube, one of which contained 0.5 ml of M/10 sodium oxalate. Platelet counts were made by the direct method described by Wintrobe(5). The plasma was separated immediately from the preoxalated tube (Tube A) by centrifugation at 2000-2500 R.P.M. for 5 minutes. The 3 remaining tubes were placed in a constant temperature, 37°C, water bath. The whole blood clotting time was determined by visual examination, timing from the filling of the first tube, which took 5-10 seconds. Clot retraction was observed after one hour and was classified as "good", as seen in the normal burro blood, when the clot was surrounded by serum and had retracted from the top of the serum or the bottom of the tube; "fair", when the clot was surrounded by serum but not retracted from the top or bottom; "slight", when a small amount of serum was visible but the clot was not surrounded, or "none", when no serum was observed. Thirty minutes after venipuncture (Tube I) and 60 minutes after clotting (Tube II), 0.5 ml of M/10 sodium oxalate was added, mixed with a glass rod, and the serum-plasma removed by centrifugation as in Tube A. Immediately following separation of cells one-stage and 2-stage prothrombin determinations were performed on all samples of plasma or serum. The modified one-stage prothrombin test(6) was the mixing of 0.1 ml of test plasma (serum), with 0.1 ml of normal fresh burro plasma treated with $BaSO_4$(7) and then adding 0.4 ml of Simplastin (Chilcott Labs.). The 2-stage prothrombin procedure described by Ware and Seegers(8) was used with the following modifications. $BaCO_3$ adsorbed burro serum was used instead of bovine serum as a source of AcGlobulin. Fibrinogen was prepared by adsorbing normal oxalated burro plasma with $BaSO_4$, precipitating with one fourth saturated $(NH_4)_2SO_4$ and dialysing against saline containing 1:200 of 1.2 M sodium citrate. Defibrination was accomplished by the addition of 25 units/ml of Upjohn's thrombin. Difco 2-stage prothrombin reagent was used as the activating mixture. A comparison of prothrombin concentration in preoxalated freshly drawn blood (Tube A) with residual prothrombin in Tube I and Tube II gave a measure of prothrombin consumption, a method suggested by Buckwalter *et al.*(9) and Langdell *et al.*(10). Recalcified plasma clotting times were determined by the method described by Ferguson *et al.*(11). Assays were done in a room held at a tempera-

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† Lt. Colonel, Veterinary Corps, United States Army.

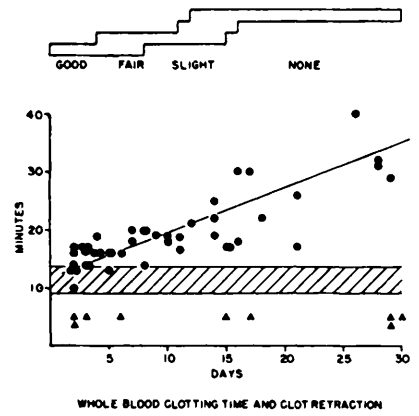

FIG. 1. Distribution of deaths represented by triangles; range of whole blood clotting time (9-13.5 min.) of pre-irradiated burros represented by shaded area; whole blood clotting times on various days after irradiation represented by dots; observations of clot retraction of blood after irradiation represented in blocked area above graph with descriptive subscripts.

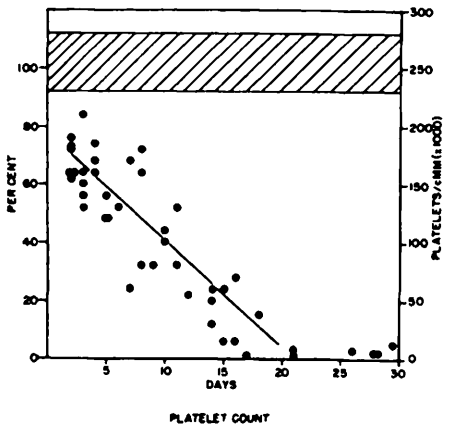

FIG. 2. Range of pre-irradiation platelet counts for burros (230000-280000/mm³) represented by shaded area. Post irradiation values on specific days of experiment represented by dots.

ture of 28°C. The straight lines on the diagrams of results were plotted by the method of least squares from all experimental data represented by dots on the diagrams and represent a trend after the initial change was noted.

Results. The *syndrome* and deaths were typical of radiation sickness in the burro (Fig. 1). The burros dying on 2nd and 3rd days had encephalitis-like symptoms. Lameness and necrotic ulcerations of mucous membranes occurred later in survivors and after 2 weeks a clinical hemorrhagic syndrome was apparent. The 3 burros living 29 to 30 days post irradiation bled through the apparently intact skin over large areas of their bodies. No gross pathological evidence of radiation damage was noted in animals dying before the 6th day. The other animals had petechiae and ecchymosis of muscles, mesentery, peritoneum and submucosa of bladder and vulva. Pulmonary hemorrhages were also observed. The lymph nodes and perinodular tissues were soft, dark and edematous without obvious hemorrhage.

There was a significant increase in whole blood *clotting times* after the 3rd post irradiation day (Fig. 1).

There was a change in the *clot retraction* rate 4 days after exposure to irradiation (Fig. 1). Gradations in quality of retraction were clearly observed.

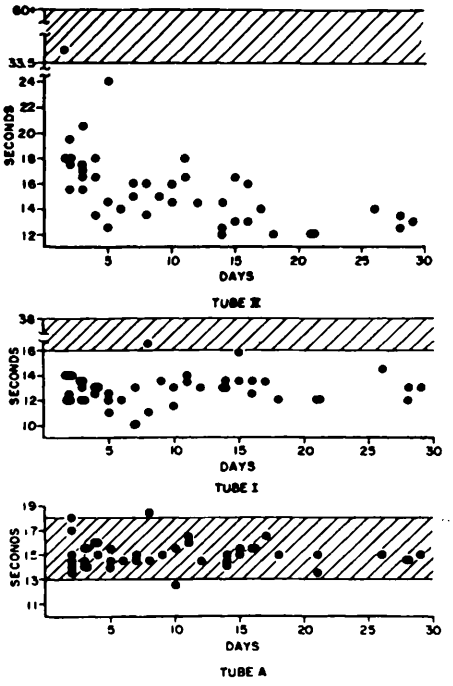

FIG. 3. Prothrombin clotting times (p.c.t.) determined by modified one-stage (Quick) method. Shaded areas represent pre-irradiation range, dots represent individual values on various days after irradiation. Normal pre-irradiation p.c.t. were: for preoxalated plasma (Tube A) 13-18 sec., for serum-plasma oxalated 30 min. after venipuncture 16-38 sec. (Tube I) and for serum plasma oxalated 60 min. after clotting (Tube II) 33.5-60+ sec.

A significant reduction of *platelets* was noted on the first examination of the blood (21 hours after irradiation began) and thereafter the decrease was progressive. The platelet counts approached zero after the 18th day (Fig. 2).

There was no significant change in *prothrombin concentration* in the blood (Tube A) by the *one-stage method* (Fig. 3). However, in the clotting samples, the one-stage technic was very sensitive to the change following irradiation. Significant changes in prothrombin clotting times were found in coagulating blood 30 minutes after bleeding (Tube I) and 60 minutes after clotting (Tube II). In many cases prothrombin clotting times in Tubes I and II were faster than in Tube A.

When measured by the *2-stage method* normal burro plasma oxalated 30 minutes after venipuncture (Tube I) (Fig. 4) there was an immediate decrease in prothrombin utilization after radiation. Late in the acute whole body irradiation syndrome as much as 90% of residual prothrombin was found.

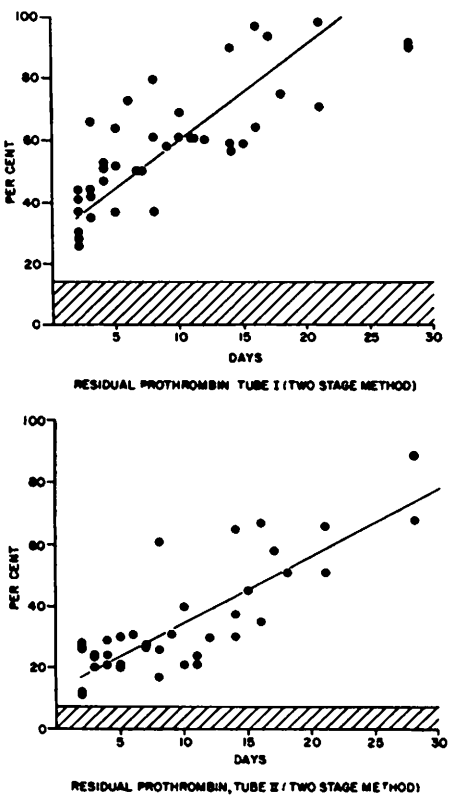

FIG. 4. Residual prothrombin determined by 2-stage method in serum plasma oxalated 30 min. after venipuncture (Tube I) and 60 min. after clotting (Tube II). Shaded areas represent pre-irradiation range (0-14% in Tube I and 0-7% in Tube II), dots represent individual values following irradiation.

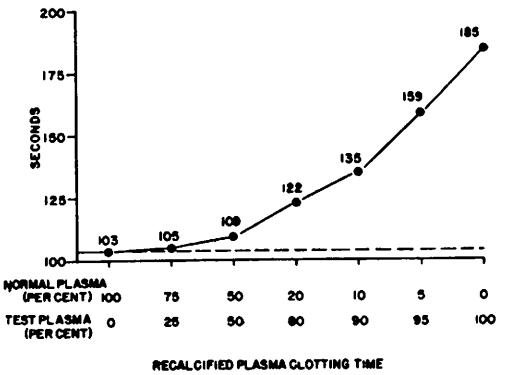

FIG. 5. Mean recalcified plasma clotting times in sec. determined by addition of 0.2 ml of $CaCl_2$ (0.02M) to 0.2 ml of plasma dilutions indicated in abscissa. Broken line represents mean of 5 normal burros. Figures and dots indicate mean values of dilutions of these normal plasmas and the plasmas of 5 individual burros alive 18 days after irradiation.

Sixty minutes after clotting, the normal burro serum (Tube II) contained 7% or less of the original prothrombin concentration. From 21 hours after irradiation and until death the residual prothrombin concentration in the serum gradually increased. Up to 85% of the total prothrombin remained in the serum one hour after clotting (Fig. 4).

There was a gradual lengthening of the recalcification clotting time (RCT) when the plasma of 5 normal burros was quantitatively diluted with plasma from 5 irradiated burros alive 15 days after radiation (Fig. 5).

Discussion. As in man(12) and other animals(13-16) with few exceptions(17,18) the whole blood clotting time was increased by fatal whole body gamma radiation of burros. In blood of burros having developed a severe clotting deficiency, there were sufficient clotting factors present for a poor venipuncture, introduction of air bubbles or undue agitations to reduce the clotting time materially. Gradation of results, not clearly observed at room temperature in 24 hours were readily detected in one hour at 37°C. Defective clot retraction

due to radiation has been previously reported (13,15,17,18).

There is a relation of platelets to normal whole blood clotting and clot retraction (18-21). The correlation of post irradiation bleeding, clot retraction and prolonged clotting times with thrombocytopenia was reviewed by Cronkite and Brecher(1) and the experimental correction of these defects by platelet transfusion is noted(22). There was no evidence of hypoprothrombinemia in our burros. With a few exceptions, these results generally agree with the findings of other studies(15,16,23).

Defects in prothrombin utilization as demonstrated (Fig. 3, 4) by both one-stage and 2-stage methods are comparable to the results reported by Jackson *et al.*(15), Cronkite *et al.* (24), Penick *et al.*(14), and Ferguson(11). Correction of this defect by platelet transfusion was noted by Dillard, Brecher, and Cronkite(20).

The correlation of the decrease in platelets with prothrombin utilization has been observed experimentally in the blood of dogs(9). Due to different methods of platelet enumerations and the empirical nature of prothrombin determination technics the degree of quantitative correlation of the exact platelet level and the occurrence of defective prothrombin utilization has not been established(9,11,15,24). In some cases there was a deficient prothrombin utilization when burro blood contained 80% of normal platelets.

The results of the 2-stage method always demonstrated a percentage utilization of prothrombin in clotting blood with routinely selected 2-stage reagents. The one-stage method frequently indicated a higher prothrombin activity in serum-plasma samples (Tubes I and II) than in fresh oxalated blood samples (Tube A). This divergence from the 2-stage results was seen in pre-irradiated (normal) blood of burros as in dogs by Langdell *et al.* (10). These workers and Alexander and Landwehr(25) suggest that it is due to a non-prothrombin factor which appeared during clotting and shortened the one-stage "prothrombin" times. With the delayed prothrombin utilization after irradiation there is an opportunity to demonstrate this factor over a longer period during clotting, confirming Ferguson's experience on dogs given Au-198(11).

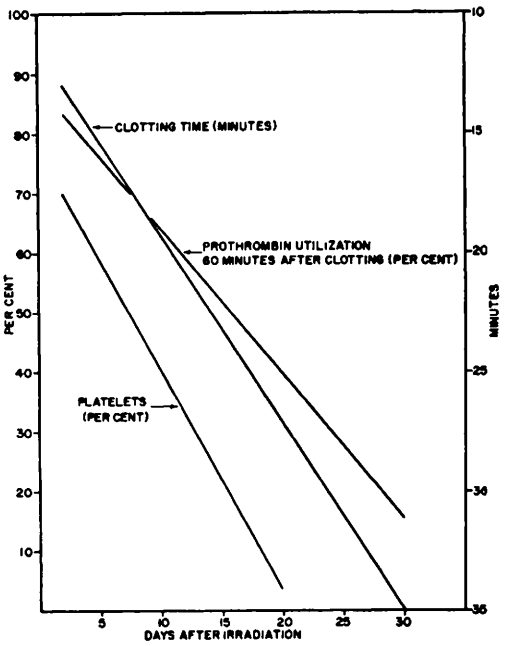

FIG. 6. Trends of reduction of platelets, decrease in prothrombin utilization and prolongation of clotting times from 2nd day after irradiation.

The burro plasma mixture recalcification test data (Fig. 5), unlike those of Ferguson's (11) radio-gold dog experiments, gave evidence of a coagulation defect, but the results must be interpreted as failure to demonstrate any circulating anticoagulant. Lewis and Ferguson (personal communication and(26)) use this test to differentiate between circulating anticoagulant and certain clotting factor deficiencies (*e.g.*, congenital hypoprothrombinemia, hemophilia, PTC lack, etc.). Whether the results of our burro tests are attributable to the thrombocytopenia or to lack of some plasma factor must await further experimental analysis as stressed by Ferguson(11).

Summary and conclusions. 1. Nine burros exposed to a lethal dose of whole body gamma radiation were observed. The burros had symptoms and pathologic changes characteristic of the irradiation syndrome. Four died during the first week and the remaining 5 died before the 30th post irradiation day. These 5 showed hemorrhagic symptoms. 2. There was a retardation of whole blood clot-

ting time. A clotting defect was demonstrated in recalcified oxalated plasmas of burros tested 2 weeks after irradiation. 3. All irradiated burros developed a severe thrombocytopenia and a lessening of clot retraction was noted on the after 4th post irradiation day, with no clot retraction after the 16th day when the platelet count was approaching zero. 4. There was a pronounced diminution of prothrombin utilization rate after irradiation. The first change was noted 22 hours after irradiation or 3 hours after the animals were removed from the exposure field. 5. There was no demonstrable circulating anticoagulant. The possible relation of platelet concentration to the clotting defects is discussed.

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Hibernation of the Lizard, *Anolis carolinensis*. (20114)

HERBERT C. DESSAUER. (Introduced by Roland A. Coulson.)

From the Department of Biochemistry, Louisiana State University Medical School, New Orleans, La.

Any attempt to evaluate the comparative biochemistry of hibernation is hindered by the sparsity of information on seasonal changes among reptiles. As part of an extensive study of reptilian biochemistry, analyses of certain metabolic indices were done each month on the lizard, *Anolis carolinensis*, to compare the effect of the seasons on animals obtained from the wild with groups kept under laboratory conditions.

In order to determine whether seasonal temperature changes are primarily responsible for the metabolic variations found, a colony of captive lizards was maintained in a windowless, constant temperature room at the average summer temperature of New Orleans,