of these substances.

The relative resistance of mice irradiated at the age of one day is not shared by mice irradiated before birth. A relatively high mortality has been observed among a small group of mice exposed to intrauterine irradiation. Mice irradiated one day before birth survive considerably longer than mice irradiated 2 or 3 days before birth. These findings suggest that profound alterations occur shortly before, during or immediately after birth which render mice less susceptible to irradiation.

Sex does not appear to affect susceptibility of mice to whole-body irradiation. A possible exception is the 90 day age group in which a suggestively greater proportion of females than males died. It is of interest that a high mortality rate (50 per cent) has been observed in a small group of pregnant female mice 90 days of age at the time of irradiation. Further investigation of the influence of pregnancy on susceptibility to whole-body irradiation is in progress.

Some of the results reported here may be helpful in the design of future experiments concerned with radiation mortality in mice (and presumably also in other species). Attention is particularly directed to the need for controlling age as a variable, and to the desirability of selecting an age such that the anticipated mortality may facilitate statistical analysis of the experimental results. The usual 30 day observation period following irradiation, which is adequate for older mice, should probably be extended to 60 days when suckling mice are being studied, and weaning time must be suitably controlled among different experimental groups.

Summary and conclusions. (1) The age at which mice are irradiated profoundly alters their resistance to a single exposure of whole body irradiation. Mortality is low after irradiation at 1 and 15 days of age, very high at 30 days, and decreases rapidly with increasing age thereafter. The possible mechanisms of this effect are discussed. (2) The response of mice to whole body irradiation is not related to body weight except insofar as weight reflects a particular age range. (3) In general, sex appears to exert no significant influence on the capacity of mice to withstand whole body irradiation.

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Influence of Concentration of Thromboplastin on Prothrombin Time of Human and Dog Plasma.* (18611)

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In the development of the one-stage prothrombin test, it was observed that on adding increasing amounts of thromboplastin, a minimum clotting time was obtained which remained constant irrespective of the excess of thromboplastin(1). In the present study, an attempt is made to correlate quantitatively the concentration of thromboplastin required for obtaining minimal clotting values when the prothrombin activity of the plasma is varied by different experimental means.

Methods. As the source of thromboplastin rabbit brain dehydrated with acetone as previously described(2) was employed. To test the potency of the material, various concentrations were prepared by mixing weighed amounts with 5 cc physiological saline and incubating the mixture at 50°C for 20 minutes. The various concentrations of thromboplastin which ranged from 1 to 250 mg in 5 cc of saline (0.02 to 5.0%) were tested on

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1. Quick, A. J., *Am. J. Physiol.*, 1936, v114, 282.

2. Quick, A. J., *J.A.M.A.*, 1938, v110, 1658.

TABLE I. Effect of Concentration of Thromboplastin on Prothrombin Time.

Thromboplastin mg*	250	200	150	100	75	50	25	10	5	2½	1
Fresh human plasma:	Prothrombin time in seconds										
With .02 M $CaCl_2$	13	13	13	13	13	14½	17	21	24	29	38
With .01 M $CaCl_2$	12	12	12	12	12	13	16	20	22	27	36
Fresh human plasma .9 cc D.† rabbit plasma‡ 0.1 cc											
With .02 M $CaCl_2$	13	13	13	13	13	16	17	20	21	28	32
With .01 M $CaCl_2$	12½	12½	12½	12½	12½	14½	16	18	19	21	18
Stored human plasma .9 cc D.† rabbit plasma‡ 0.1 cc											
With .02 M $CaCl_2$	8	8	8½	9	9½	10	10½	13	16	17	18½
With .01 M $CaCl_2$	8½	8½	9	9	9½	10	10	13	16	17	18½
Fresh dog plasma:											
With .02 M $CaCl_2$	6	6	7	7½	8	9	11	12½	14	16	22
With .01 M $CaCl_2$	6	6	7½	8	9	10	12	13½	17	21	29
Dog plasma (Dicumarol) I§	12	12	12	12	12	13	13½	14	15	16	17½
Dog plasma (Dicumarol) II§	25	25	25	25	25	27	28½	31	36½	38½	41½
Dog plasma (vit. K deficiency)§	20	20	20	20	20	21½	23	25	27½	34	40

* Amount of thromboplastin mixed with 5 cc saline solution.

† Deprothrombinized.

‡ The prothrombin was removed with tricalcium phosphate according to the procedure of Quick and Stefanini(5).

§ Only .02 M $CaCl_2$ was used.

oxalated human and dog plasma according to the one-stage prothrombin test(2). In the collection of the blood, silicone coated glassware was employed to avoid the changes that occur when blood is in contact with glass. The thromboplastin requirement of both fresh and stored human oxalated plasma were studied. For the latter, the blood was put in glass test tubes and kept in the refrigerator at 4°C for 2 to 4 days. In testing the prothrombin activity of stored plasma, the labile factor which disappears on storage had to be replaced. This was done by adding 1 volume of deprothrombinized rabbit plasma, prepared by adsorption with tricalcium phosphate, to 9 volumes of stored plasma(3). Rabbit plasma has an exceedingly high concentration of labile factor. For a control, deprothrombinized rabbit plasma was added to fresh human plasma, and the requirement of thromboplastin compared with unmixed fresh human plasma. Likewise normal dog plasma was compared with that obtained from animals that had been treated with Dicumarol.

Results. From the data in Table I, it can be seen that the concentration of thromboplastin obtained by mixing and extracting 75 mg of dehydrated rabbit brain with 5 cc physiological saline solution, yielded maximum activity when tested on fresh human oxalated plasma. In contrast to this, stored human plasma with its labile factor restored by means of added deprothrombinized rabbit plasma, required 200 mg of thromboplastin in 5 cc of saline. Furthermore, the minimum prothrombin was 8 seconds in contrast to the 12 seconds of fresh human plasma. The addition of deprothrombinized rabbit plasma to fresh human plasma did not shorten the prothrombin time but actually prolonged it slightly, and did not alter the thromboplastin requirement.

Normal dog plasma required 200 mg of thromboplastin in 5 cc saline for a minimum prothrombin time of 6 seconds, but when the prothrombin level was reduced by Dicumarol, 75 mg was sufficient to obtain the shortest constant prothrombin time.

It was noted that 0.02 M $CaCl_2$ gave the shortest prothrombin time for all plasmas studied except fresh oxalated human plasma. For the latter, 0.02 M $CaCl_2$ had a slight but unmistakable depressing action whereas 0.01 M did not. This was consistently observed only when blood was collected and kept in silicone-coated glassware. When blood was in contact with glass this inhibitory action of calcium was lost.

3. Quick, A. J., and Stefanini, M., *J. Lab. and Clin. Med.*, 1948, v33, 819.

Discussion. When human oxalated plasma is stored in glass, its prothrombin time becomes prolonged because of the disappearance of an agent which we have designated labile factor and which is probably the same as Factor V of Owren, the Ac-globulin of Seegers, the accelerator by Fantl, and the thromboplastin co-factor of Honorato. On restoring the labile factor by the addition of deprothrombinized rabbit plasma, which has a high concentration of this agent, the prothrombin time becomes 8 instead of 12 seconds, the normal for fresh human plasma. This finding can be interpreted as being due either to an increase in active prothrombin or to the formation of an agent which accelerates the conversion of prothrombin to thrombin as Alexander and his associates maintain(4). If such an accelerator exists, it is not identical with the labile factor, because the addition of the latter to fresh plasma does not increase prothrombin activity.

Aged human plasma with the labile factor restored requires definitely more thromboplastin for optimum prothrombin activity than does fresh plasma. This is independent of the labile factor since an excess added to fresh plasma has no effect. Stored human plasma behaves like fresh dog plasma in respect to thromboplastin requirement. When, however, the prothrombin level of dog plasma is reduced by Dicumarol or vitamin K deficiency, less thromboplastin is needed to obtain the constant minimum prothrombin time. This suggests that the thromboplastin requirement varies with the concentration of prothrombin. If this conclusion is correct, the increase in prothrombin activity in stored human plasma may be explained by an actual increase in active prothrombin. Quick and Stefanini(5) have postulated that the prothrombin in human blood is partly in an active form and partly in a precursor state which they designated prothrombinogen.

Summary. The amount of thromboplastin required for optimum prothrombin activity increases when human plasma is stored and the lost labile factor replaced. It decreases in dog plasma when the prothrombin level is reduced by means of Dicumarol or vitamin K deficiency. It is concluded that a direct relationship exists between the concentration of prothrombin and the amount of thromboplastin, for obtaining a minimal constant prothrombin time.

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Endothelial Damage by X-Rays Disclosed by Lymph Fistula Studies.* (18612)

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Studies of the rate of disappearance of varied substances from the blood of rabbits and mice(1) led to the assumption that capillary permeability is greatly increased in these species by exposure to large (LD-50) doses of X-rays. Tagged plasma, homologous and heterologous erythrocytes, and other tagged large-molecular substances when injected into the blood disappeared from the blood stream of X-rayed rabbits and mice (2 to 14 days after irradiation) faster than from that of normal control animals(1,2). In order to bring direct evidence for heightened capillary permeability and to ascertain its

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