There are a number of fundamental differences between these experiments and earlier studies. The problem of antibody development is avoided by use of rats whose blood and tissue compatibilities are so complete that parabiotic union can be accomplished and maintained. None of the donors was used more than once.

Experiments at this laboratory in which rats received 4 ml of homozygous plasma per day for 2 weeks, have shown that plasma transfusion does not cause a reduction in red cell production. Others have made the same observation(5).

The inhibition of red cell iron turnover by increasing the red cell volume may be useful in studies of control of erythropoiesis.

Summary. (1) A profound decrease in the red cell iron turnover occurred after experimental increase in the red cell volume of rats. (2) A major portion of the tracer was found in the liver at necropsy.

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Species Specificity of Thromboplastin: Role of the Cothromboplastin Reaction (19258)

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It has been recognized for a long time that species differences may influence the coagulative activity of thromboplastin (tissue extract) on plasma. Thus Quick(1) found that mammalian thromboplastin was relatively inactive with respect to the coagulation of avian plasma and avian thromboplastin was similarly inactive with respect to mammalian plasma. Thromboplastin from rabbit brain was highly active with respect to human plasma but this was not true of thromboplastin from guinea pig brain. Recently Burstein(2) has reported that the activity of mammalian thromboplastin with respect to avian plasma could be greatly increased by the addition of mammalian serum to the reaction mixture: for a maximal effect addition of 0.1 ml of undiluted serum to the 0.3 ml reaction mixture of the prothrombin time test was needed. We have found that normal serum greatly increases the activity of thromboplastin with respect to dicumarol plasma. This effect will occur with highly diluted serum (1:100) provided that the serum and thromboplastin are allowed to react for a brief period before the plasma is added. We have presented evidence (3-5) that this reaction, which we have called the cothromboplastin reaction, is attributable to a specific factor, cothromboplastin, which is present in both plasma and serum and is decreased by dicumarol much more rapidly than is prothrombin. It seemed worth while to determine whether cothromboplastin might be involved in the property which homologous serum has of increasing the activity of thromboplastin with respect to heterologous plasma.

TABLE I. Effect of Dilute Serum on Reactivity of Thromboplastin and Plasma. Thromboplastin pretreated for 3 min with calcium and material tested, then clotting reagent (plasma) blown in. (With important exceptions noted.)

Material tested	Clotting time, sec.
A. Rabbit thromboplastin and chicken plasma	
Saline solution	86
Normal rabbit serum 1-100	20
1-100	47*
1-100	>240 (fibrinogen-acacia instead of chicken plasma)
1-10	14
Dicumarol rabbit serum 1-100	81
1-10	76
B. Guinea pig thromboplastin and human plasma	
Saline solution	76
Guinea pig serum 1-100	15
1-100	37*
1-100	>120 (fibrinogen-acacia instead of human plasma)
Human serum 1-100	85
C. Chicken thromboplastin and rabbit plasma	
Saline solution	68
Chicken serum 1-100	15
1-100	50*
1-20	11
1-20	>1800 (prothrombin-free chicken plasma instead of rabbit plasma)
Rabbit serum 1-100	67
1-20	60

* Not pretreated.

Method. Thromboplastin was prepared from the brains of rabbits, guinea pigs and chickens, by the procedure described by Quick (6). Each brain was dehydrated with acetone. The method of testing thromboplastic activity was essentially the Quick prothrombin time procedure with one additional step. This was a brief period of exposure of the thromboplastin to dilute serum in the presence of calcium, after which the plasma (instead of the calcium as in the usual Quick prothrombin time procedure) was blown in and the clotting time measured. When dicumarol plasma was employed as clotting reagent we have referred to this procedure as a one-stage cothromboplastin assay. It was suitable for testing materials which contain no appreciable quantity of prothrombin; for testing plasma a different procedure was used(3). The serum specimens tested were obtained by allowing blood to clot in small glass tubes and then to stand 24 hours at room temperature. The specimens of plasma were used within 2 hours after the blood had been drawn. The reproducibility of the reported clotting times was of the high order well known to be obtained with the prothrombin time by persons experienced with this test; accordingly the differences in the representative experiments reported are quite gross. One hundred eleven clotting experiments were done in this study.

Observations. Pretreatment with a 1:100 dilution of rabbit serum for 3 minutes greatly increased the activity of rabbit thromboplastin with respect to chicken plasma (Table IA). This brief interval of pretreatment was required for the reaction since there was much less effect if it was omitted. No significant quantity of thrombin formed during this period however, as would be expected since the serum was essentially free of prothrombin. (This was checked by the two-stage method.) Serum from a dicumarolized rabbit had virtually no effect on the activity of the thromboplastin. (The dicumarol serum had somewhat more labile factor activity than the normal serum as judged by shortening of the prothrombin time of aged plasma.)

The relative inactivity of guinea pig thromboplastin with respect to human plasma was corrected by dilute guinea pig serum but not by human serum (Table IB). Similarly, chicken thromboplastin was rendered more reactive with respect to rabbit plasma by

TABLE II. Thromboplastin from Normal Rabbit Brain Compared to That from Dicumarolized Rabbit Brain. Procedure as in Table I, using dicumarol rabbit plasma as clotting reagent. Plasma from same rabbit as dicumarol serum used in Table I.

Material tested	Clotting time, sec. Normal thromboplastin	Clotting time, sec. Dicumarol thromboplastin
Saline sol.	64	190
Normal rabbit serum 1-100	15	15
Dicumarol rabbit serum 1-100	50	

treatment with dilute chicken serum but not by rabbit serum (Table IC). In order to be effective, the serum must be homologous to the thromboplastin, not to the plasma.

Thromboplastin from the brain of a dicumarolized rabbit has been reported to be relatively inactive with respect to dicumarol plasma(7-9). Pretreatment with dilute normal homologous serum removed this difference and greatly shortened the prothrombin time of dicumarol plasma (Table II). These data are included for the purpose of comparing the effects obtained with normal heterologous and with dicumarol homologous plasmas.

Summary. Species specificity of thromboplastin is largely eliminated by brief treatment of the thromboplastin with dilute homologous serum. This appears to be an example of the cothromboplastin reaction.

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Ineffectiveness of Parenteral Pyroxidine in Relieving or Preventing the Egg White Syndrome in the Rat.* (19259)

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The report of MacKay and Barnes(1) that signs of the egg white syndrome were cured in the rat by the injection of vit. B_6 when corn oil was included in the ration, has been interpreted by Sherman(2) in a recent review, to offer evidence that ". . . pyridoxine and essential fatty acids act mutually to enhance or replace biotin."

Salmon and Goodman(3) observed that the severity of the skin lesions in the egg white syndrome were decreased and their character altered somewhat by the presence of linseed oil, butter fat or hydrogenated cottonseed oil in the diet. They attribued this effect to the unsaturated fatty acids which were supplied by the fats. On the other hand, Nielson and Elvehjem(4,5) found that neither corn oil (5 or 10%) nor pyridoxine (100 to 125 μg orally, daily) offered protection against spectacle eye condition or paralysis in their rats on egg white rations. However, as there was the possibility that the effectiveness of pyridoxine in the study of MacKay and Barnes might have been attributable to their use of the parenteral method of administering the vitamin, this was explored.

Experimental. The basal ration had the following percentage composition: egg white

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