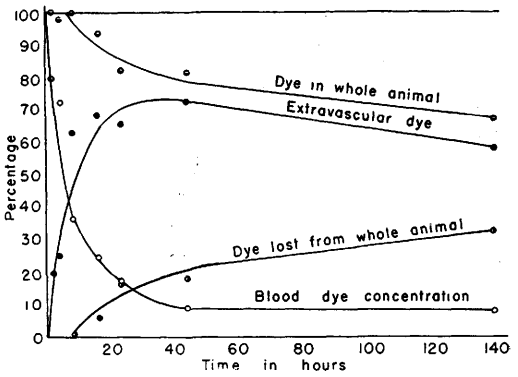

FIG. 1.

plained by the presence in blood of material which yields color by carcass analysis but not by blood analysis. This is highly improbable and, moreover, no blue color was observed in Somogyi precipitates of blood. The conclusion appears justified that the dye in the carcass not accounted for by blood analysis is in the extravascular spaces.

Discussion. Sellers *et al.*(7) have reported serum Evans blue/serum protein concentration ratio as a function of time up to 70 hours after intravenous injection into rats of 20 mg of Evans blue. They make no mention of employing anesthesia except for terminal sampling. If serum protein concentration is assumed to remain constant, their curve can be considered a blood dye concentration curve, and in fact their curve is almost identical with that for blood in Fig. 1. This similarity suggests that the present findings were not greatly influenced by the ether anesthesia.

Summary. 1. A method is described for determination of Evans blue dye in various animal tissues. In brief it consists of (1) homogenization of tissue in a concentrated solution of urea, (2) splitting of the Evans blue-protein complex and partial precipitation of protein and chromogens with acetone, and (3) complete precipitation of chromogens by addition of Somogyi reagents. 2. The method has been employed to follow the fate of the dye after intravenous injection into rats. After such administration of 12.5 mg of Evans blue, about 90% of the injected dye apparently left the blood but only about 15% could not be recovered from the body in 40 hours. At 140 hours after injection about 67% of the injected dye could still be recovered from the body.

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Plasma Prothrombin Time and Hematocrit Values of Blood of Dairy Cattle.* (22156)

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Hemostasis is a complicated mechanism of blood coagulation composed of many coordinated reactions(1-3). According to Quick (2), coagulation of blood occurs in 3 stages, none of which are complete when considered individually. These stages must be combined in a definite arrangement before a complete explanation of hemostatic mechanism can be offered.

Determination of plasma prothrombin time

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has become essential in diagnosing, treating, and studying hemorrhagic diseases in humans (4). Since prothrombin level of blood in animals is decreased by dicumarol present in damaged sweet clover(5), in avitaminosis-K (6), and in other hemorrhagic diseases(7,8), normal prothrombin time is of considerable value in veterinary medicine. Pritchard *et al.* (9) found clotting time of blood to be lengthened when soybeans extracted with trichloroethylene were fed to cattle, but plasma prothrombin time was not lengthened appreciably. A direct correlation between plasma prothrombin time and blood clotting time was not found. This emphasizes the need for both plasma prothrombin time and blood clotting time in research with hemorrhagic diseases. A normal prothrombin time (adequate prothrombin in blood) is possible with prolonged clotting time. In this case it appears that deficiency of any one of the items (labile factor, fibrinogen, calcium, or thromboplastinogen) described by Quick(2) in blood coagulation may be present. On the other hand, prothrombin time may be prolonged with normal coagulation time. Normally excess of prothrombin is present in plasma; therefore hypoprothrombinemia must be marked before coagulation time is lengthened. Prothrombin time of plasma of sheep has been reported (10). There is, however, little information available on other ruminants. Prothrombin time of cattle is herein reported.

Methods. The first phase of this investigation was made possible through cooperation of several dairy farmers. Cows had been fed a standard dairy ration in addition to pasture grazing. Sweet clover pasture or clover hay had not been fed the past year. In the second phase, calves from another experiment (11) were divided into 3 groups. Group I calves were fed a standard calf-starter mix (basal); Group II calves were fed basal ration plus 5 trace minerals (Fe, Cu, Co, Mn, and Zn); and Group III calves were fed basal ration, trace minerals, fat-soluble vitamins, water-soluble vitamins, calcium, magnesium, phosphorus, and aureomycin. Blood samples were obtained from jugular vein using a mixture of ammonium and potassium oxalate(12) as the anticoagulant. In carrying out the one-stage plasma prothrombin time of Quick(2) 0.1 ml of plasma was pipetted into 7 by 100 mm pyrex test tubes. To the test tubes containing plasma, 0.1 ml of Bactothromboplastin† was added. Blood plasma and thromboplastin were mixed and placed in a thermostatically controlled water bath at 37°C. After 5 minutes, 0.1 ml of 0.02 M calcium chloride solution was blown forcibly from a pipette into the mixture and agitated by shaking gently in water bath. A stop watch was started simultaneously with addition of calcium chloride solution. At the end of approximately 10 seconds the tube was wiped free of water and held so that its contents were between the eye and a bright source of light. By rotating the test tube clockwise, it was possible to determine immediately when the solution began to clot and adhere to the wall of the test tube. Prothrombin time reported is that interval between addition of 0.02 M calcium chloride solution to the plasma-thromboplastin mixture and the first evidence of formation of a discernible clot or gel. Hematocrits were obtained by centrifuging 1 ml of oxalated blood from cows in Wintrobe hematocrit tubes for 30 minutes at 3000 rpm(13). Results were read directly from tubes as % packed erythrocytes.

TABLE I. Average Plasma Prothrombin Time Values of Matured Cows.

Breed	Herd No.	No. animals	Prothrombin time (sec.)
Jersey	1	8	20.63 ± 2.33*
	2	10	20.60 ± 3.43
	3	18	25.83 ± 2.54
	4	22	22.77 ± 3.78
	5	8	19.25 ± 1.25
	6	23	27.04 ± 5.28
	Total	89	Avg 23.74 ± 4.61
Ayrshire	5	9	20.67 ± 4.21
Guernsey	5	8	20.38 ± 2.27
Holstein	5	9	22.89 ± 2.03
All breeds	Total	115	Avg 23.20 ± 4.42

* Stand. dev.

Results. Plasma prothrombin time was obtained on 115 matured dairy cows and 90 dairy calves from 2 to 24 weeks of age. Table I shows that average prothrombin

† Difco Laboratories, Detroit, Mich.

time for 6 herds of Jerseys was 23.74 ± 4.61. Analysis of variance showed that there was a highly significant difference (1% level) among herds. By calculating the significant mean difference among Jersey herds, the difference between herds 3 and 4 reached the 5% level of significance, differences between herds 1 and 3 and between 2 and 3 reached 1% level, and differences between herds 1 and 6, 2 and 6, 3 and 5, 4 and 6, and 5 and 6 reached 0.1% level of significance.

In analyzing data statistically of 4 breeds of cows in Table I, the F-value reached the 5% level of significance. The difference between the means for Jerseys and Guernseys was significant at the 5% level. The difference between Jerseys and Ayrshires approached the 5% level of significance.

According to Quick(2) plasma prothrombin time should be determined within 30 minutes after blood is collected, if accuracy is desired. A comparison was made of plasma prothrombin time values determined at 0.5, 2.5, and 3.5 hours after bleeding on 10 animals in herd 2. At these intervals prothrombin time values were 20.6 ± 3.43; 20.2 ± 3.80; and 21.0 ± 2.75, respectively. These values were of no significance statistically when compared with 0.5 hour values.

Prothrombin time was obtained on plasma samples from 20 newborn calves prior to nursing. A clot or gel could not be detected in the test tube as usually observed. These test tubes were then placed in temperature controlled water bath for 2 hours, with periodic checking for clot formation. During this 2 hour period a clot did not form.

Table II shows plasma prothrombin time values of heifer calves 2 to 24 weeks old. There was no significant difference in prothrombin time values associated with age.

Plasma prothrombin time values of bulls and heifers were pooled to evaluate breed effects involving 90 bleedings. Average prothrombin time for Holstein calves was 25.15 ± 4.63 as compared with 24.55 ± 4.35 for Ayrshires. There is no significant difference between the values for Holstein and Ayrshire calves. Evidently rations did not alter prothrombin time values of calves significantly.

TABLE II. Average Plasma Prothrombin Time Values of Holstein and Ayrshire Calves at Various Ages.

Age (wk)	No. animals	Prothrombin time (sec.)	Range (sec.)
2	11	26.91 ± 4.51*	18-32
4	11	23.00 ± 5.53	16-30
6	12	25.92 ± 4.85	22-35
8	14	25.50 ± 3.63	20-33
12	13	24.85 ± 4.21	20-34
18	8	23.38 ± 4.47	20-30
24	4	26.50 ± 4.51	18-30
Total	73	Avg 25.11 ± 4.54	16-35

* Stand. dev.

Average prothrombin time for control calves was 24.80 ± 3.88, for trace mineral supplemented calves 24.61 ± 4.23, and for the vitamin-supplemented group 24.83 ± 5.27. Trace minerals and other nutrients in addition to aureomycin did not delay or shorten prothrombin time of blood plasma.

Plasma prothrombin time values for Holstein and Ayrshire calves were pooled giving an average of 23.56 ± 3.96 seconds for bull calves and 25.10 ± 4.56 for heifers. These averages show a lower prothrombin time for bull calves than for heifers; however, in analyzing the data with the t-test no significant difference statistically was found between sexes.

In Table III the average hematocrit values for 92 matured cows are given, average 35.08 ± 3.30%. There is an absence of breed effects but herd effects within the Jersey breed are significant at the 5% level. In calculating the significant differences among Jersey herds, a significant difference (5% level) was

TABLE III. Average Hematocrit Values of Matured Cows.

Breed	Herd No.	No. animals	Packed RBC (%)
Jersey	1	8	32.50 ± 1.41*
	2	10	35.05 ± 2.75
	3	18	35.56 ± 3.79
	4	22	35.45 ± 3.82
	5	8	36.13 ± 3.42
	Total	66	Avg 35.14 ± 3.48
Ayrshire	5	9	34.67 ± 2.50
Guernsey	5	8	36.00 ± 3.46
Holstein	5	9	34.22 ± 2.74
All breeds	Total	92	Avg 35.08 ± 3.30

* Stand. dev.

found between herds 1 and 3, 1 and 4, and 1 and 5.

Discussion. In Table I differences in plasma prothrombin time values among herds of Jersey breed and between Jersey and Guernsey breeds, that are of statistical importance, are probably influenced by rations. It has been shown in chickens(14) that feeding of terramycin lengthens blood clotting time. Other factors altering coagulation of blood are deficiency of vit. K(13), liver damage(13), poisonous plants(15,16), bacterial toxins(17), and halogenated hydrocarbons (18).

The plasma prothrombin time values for 10 animals fed trichloroethylene extracted soybeans(9) ranged from 21 to 28 sec., average 23.35. Averages reported in this paper are 23.20 on 115 matured cows and 24.82 on 90 calves. In no instance could the "incipient web of fibrin" be observed with blood plasma from cattle, as described by Quick (2) using human plasma, regardless of the number of variations placed upon the available light sources.

Numerous attempts were made to determine the plasma prothrombin time for calves immediately after parturition and before nursing. In all cases a clot did not form in the plasma with 0.02 M calcium chloride solution within 2 hours after mixing the contents of the test tubes. Sanford *et al.*(19) have shown that the % of prothrombin in blood plasma is slightly reduced in human infants. After birth the % of prothrombin is markedly reduced but the usual prothrombin time values are normally reached in 6 to 7 days. There is a similar trend in plasma prothrombin time of newborn calves. Greaves(20) and Bodansky(21) have attempted to explain the factors involved.

In some instances, it was found that a clot could not be obtained using a given plasma sample. Upon checking the time which had elapsed since the calcium chloride had been put into solution, it was found that 48 hours after preparation it was no longer effective in producing a clot in the mixture of plasma and thromboplastin. When a fresh solution of calcium chloride was prepared, a prothrombin time value well within the range of other samples that had been similarly processed was obtained. According to Guerrant(22) a change in the concentration of ionized calcium may have lengthened the prothrombin time. Wiggers(23) states that there is a maximum and minimum concentration of calcium chloride for the determination of plasma prothrombin time. When the concentration is not within this range, the prothrombin time is increased.

Summary. 1. The average plasma prothrombin time value of 115 matured dairy cows was 23.20 ± 4.42 and for 90 dairy calves 24.82 ± 4.47. The differences in the prothrombin time values among 6 herds of Jersey cows were highly significant (1% level) and significant (5% level) among breeds between Jerseys and Guernseys. The principal factor contributing to these effects was ascribed to rations. Age, breed, ration, and sex did not alter the prothrombin time values of dairy calves. 2. The holding of the blood plasma samples for 2.5 and 3.5 hours after collection of blood samples did not change the prothrombin time significantly when compared with the 0.5 hour values. 3. The plasma prothrombin time of newborn calves, prior to nursing, could not be determined since a clot or gel did not form in the blood plasma-thromboplastin mixture after the addition of calcium chloride. This finding corresponds with the observations made with blood plasma from human infants during the first week of life. 4. The average hematocrit value of 92 dairy cows consisting of Jersey, Guernsey, Ayrshire, and Holstein breeds was 35.08 ± 3.30. Breed differences in hematocrit values were not present; however, the differences among herds of the Jersey breed were significant at the 5% level.

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Hematogenous Pyelonephritis in Rats. II. Production of Chronic Pyelonephritis by *Escherichia coli.** (22157)

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Development of chronic pyelonephritis in man is obscure because usually the disease is examined only in its end stages. The present experiments were undertaken therefore to provide an experimental model for a controlled study of processes which culminate in chronic pyelonephritis. A major objective was to observe effects of chronic infection on the kidney in the absence of hydronephrosis or other structural abnormalities. We described previously a simple, non-surgical technic for producing acute hematogenous phylonephritis in rats, involving 2 steps; injection of *Escherichia coli* into blood, and massage of kidneys through intact body wall(1). The resulting disease was similar to acute pyelonephritis without manifest hydronephrosis in man, but self-limited, and subsided in approximately 6 weeks. To produce chronic pyelonephritis, rats from this study were subjected repeatedly for several months to bacterial injection and renal massage. Special interest was directed to the following problems: 1) relationship of chronic pyelonephritis to vascular disease and hypertension, 2) progression of chronic renal inflammation after cessation of active infection.

Materials and methods. Sixty-one male Sprague-Dawley rats, weighing 150 to 200 g each, were divided into 5 groups of 12.† All were fed rat chow‡ and tap water *ad lib.* Treatments for each group are described in Table I. During the first 30 weeks, urine from rats in each group was obtained peri-

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† Group V consisted of 13 rats.

‡ Uncle Johnny Mills, Houston, Texas.