nique of Saffran and Schally was utilized for detection of factors affecting the release of growth hormone *in vitro*. Pituitary stalk-median eminence extracts of porcine and bovine origin were shown to contain a growth hormone releasing factor (GRF).

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Blood Coagulation and Hemostasis in Thoroughbred Horses.* (30139)

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Relatively few observations concerning the hemostatic mechanism of the horse are available and conflicting results have been reported(1-4). As part of a current investigation of epistaxis in race horses it was possible to evaluate coagulation factors, platelet number and adhesiveness and bleeding time in a group of thoroughbred horses. The effect of exercise on several coagulation factors was also measured.

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Materials and methods. The horses studied were all in active training during the 1964 racing season. Coagulation studies were done on 20 horses: 13 normal and 7 "bleeders." The latter had history of at least one episode of epistaxis during or after running, but were otherwise well. Their ages ranged from 2 to 7 years (mean 3.5 years). The group included 6 gelding, 6 mares and 8 stallions. Bleeding times were done on 9 additional normal thoroughbreds.

Blood was collected from the jugular vein through an 18 gauge needle into plastic

TABLE I. Results of Studies on Thoroughbred Horses.

Test		No. of horses studied	Mean value	Range
Clotting time (glass)	(min)	15	11.8	8– 17.5
" " (silicone)	"	15	17.8	13– 25
Recalcification time	(sec)	15	128	84–180
Kaolin partial thrombo-plastin time	"	17	59	50– 75
Prothrombin time	(%)*	20	190	143–255
Prothrombin-proconvertin	"	15	180	121–400
Prothrombin assay	"	15	152	126–195
Factor V "	"	15	474	275–720
" VIII "	"	20	132	84–160
" IX "	"	20	273	116–688
" XI "	"	20	56	24–108
" XII "	"	15	76	50–103
Fibrinogen	(mg %)	19	195	105–316
Platelet count	(10^3/mm^3)	5	89	78–103
Platelet adhesiveness	(%)	5	39	28– 51
Bleeding time	(min)	17	8.3	7– 10.5

* Results reported in % of normal human mean (see text).

syringes. Whole blood clotting times were performed in glass and in siliconized tubes by the Lee-White method(5) in a 37°C portable water bath at the stables. Blood for the remainder of the coagulation studies was collected in citrate-citric acid anticoagulant (9 parts blood to 1 part anticoagulant)(6) in plastic centrifuge tubes, cooled in ice and centrifuged promptly. Aliquots of plasma in plastic tubes were either kept in ice or quick frozen and maintained at —20° until assayed. The recalcification time of plasma was done at 37°C using equal parts of plasma and 0.025 molar calcium chloride. Other studies included the kaolin partial thromboplastin time (KPTT)(6), prothrombin time of Quick(7) using Simplastin (Warner-Chilcott), prothrombin and proconvertin test (P&P) and prothrombin assay(8) and factor V assay(5). For the latter test factor V deficient substrate plasma was prepared by ageing normal human oxalated plasma at 37°C until the prothrombin time was greater than 50 seconds. Standard Normal Plasma (SNP-Dade Reagents, Inc.) was used to construct standard curves for the prothrombin complex tests and values are expressed in per cent of the SNP standard.

Assay of factor VIII (anti-hemophilic factor) was based on the correction of the kaolin partial thromboplastin time of human factor VIII deficient plasma(9). Assays for factor IX (plasma thromboplastin component), factor XI (plasma thromboplastin antecedent) and factor XII (Hageman factor) were performed in an identical manner using specific human deficient plasmas as substrate in the assays for factors IX and XII. An artificial factor XI deficient substrate was prepared from bovine plasma adsorbed with Filter-cel (Johns-Manville) 6 mg/ml for 10 minutes. Standard curves were obtained for each of the factors (VIII, IX, XI and XII) using dilutions of frozen normal human plasmas from individuals previously shown to represent the mean normal value for the specific factor being assayed.

Fibrinogen was determined by the method of Ratnoff and Menzie(10). Bleeding times were performed by making an incision 1 mm deep and approximately 10 mm long on the inner aspect of the lower lip using a no. 15 Bard-Parker blade fixed in a small disc of plastic in a manner that allowed only 1 mm of the blade to project. This apparatus was easily concealed from the horses' view prior to the incision. Platelet adhesion was measured by the method of Salzman(11) modified so that both samples were obtained from the same venipuncture. Platelet counts were done by phase microscopy.

Results. The results of clotting tests, coagulation factor assays, bleeding times and platelet studies are reported in Table I. The

TABLE II. Effect of Exercise on Prothrombin Time, Fibrinogen, Factors VIII, IX, XI. Results are mean values of 5 horses.

	Before exercise	After exercise	% Change from baseline
Prothrombin time	204%	245%	+20
Fibrinogen	183 mg%	252 mg%	+38
Factor VIII	159%	139%	−12
" IX	386%	264%	−32
" XI	29%	25%	−13
Platelet adhesiveness (3 horses)	37%	51%	+27

coagulation tests in the 7 "bleeder" horses did not differ significantly from the mean values of normal horses and are therefore included in the total normal results.

Clotting time in glass was slightly longer than the normal human range and is similar to previous reports. Both recalcification time and the kaolin partial thromboplastin time appear to be within the normal human range. The mean results for tests measuring factors in the prothrombin complex were higher than human values. Factors VIII and IX were also higher than human values, whereas factor XI was 56% and factor XII was 76%. The mean fibrinogen concentration was 195 mg%.

The bleeding time on 17 normal horses ranged between 7 and 10.5 minutes, mean 8.3 minutes. The mean platelet adhesiveness of 5 normal horses was 39%. Platelet counts on these horses revealed a mean of 89,000/mm^3.

Five horses were studied before and after their scheduled morning exercise which consisted of running from one-half to five-eights of a mile. The post-exercise samples (Table II) were obtained within 30 to 60 minutes after exercise, usually after the animal had been "cooled down" by walking.

Discussion. A smaller difference between glass and silicone clotting times was noted in horses than in humans and may be related to lower levels of factors XI and XII in horse plasma. Although results of tests of overall coagulability (recalcification time and KPTT) were similar in horse and man, several specific assays differed significantly. Tests measuring the "prothrombin complex" (prothrombin and factors V, VII and X) revealed values greater than those of humans. Factor V levels were particularly elevated as they are in several other animal species (12). Factor VIII was within the range reported for humans in contrast to elevated factor VIII levels reported in several mammals(12). Moderate elevation of factor IX was noted (273%) whereas values for factor XI (56%) and factor XII (76%) were lower than those in man. The relatively low levels of factor XI were previously noted by Botti and Ratnoff(13). They utilized horses to prepare factor XI deficient substrate plasma and reported concentrations of 35 to 70% of normal human plasma.

Assay of horse plasma following exercise did not reveal the rise in factor VIII seen in humans(14,15) although a moderate increase in fibrinogen was detected. The present study does not reveal whether this represents a true species difference. Egeberg has shown that repeated exercise in humans leads to decreased responsiveness of factor VIII (16); a similar mechanism may explain the absence of increase in the horses studied since they were in training and running frequently.

The relatively low platelet counts are similar to previously reported values for thoroughbreds(2) but contrast with higher values reported for farm horses(17). Platelet adhesiveness expressed in per cent adhesive platelets reveals a mean nearly identical to that reported for humans(11).

Bleeding time studies as described have not been previously reported in horses and appear to be comparable to results of bleeding times in humans by the method of Borchgrevink and Waaler (modified Ivy technique) (18).

Summary. Coagulation studies, bleeding times, platelet counts and platelet adhesiveness were measured in a group of thoroughbred horses. When compared to normal human values the horse had elevated levels of the prothrombin complex factors and factor IX, equal levels of factor VIII, and low levels of factor XI and factor XII. Exercise did not alter the level of factor VIII. Bleeding time and platelet adhesiveness were comparable to human values, although the horses' platelet counts were relatively low.

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Indocyanine Green-Plasma Half Time Clearance (T ½) in Normal Beagles.* (30140)

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Indocyanine green (Cardio-Green®)† when given in a single injection has been reported to be an excellent and relatively simple test for detecting liver damage in humans and dogs (1-4). This tricarbocyanine dye when injected intravenously is rapidly bound to the plasma albumin (95%) and cleared exponentially during the first 20 minutes(1,2). This clearance is accomplished almost exclusively by the liver as shown by Wheeler(5), who recovered 96% of the total injected dye in the bile of dogs. There is also evidence that indocyanine green (ICG) is equal, if not superior to, Bromsulphalein (BSP) when used to assess liver function(3,4,6).

The following study was undertaken to establish normal values for ICG clearance when the dye was given in a single injection to normal adult beagles.

Animals. Eight purebred beagles, 4 males and 4 females, were used in this study. Weights ranged from 8.9 to 13.4 kg with a mean of 11.2 kg. Ages ranged from 4 to 6 years with a mean of 5 years and 2 months.

The animals were housed in comfortable kennels, were fed a commercial ration once a day and water was available at all times. Food was withheld for 24 hours prior to injection with ICG.

The animals were divided into 2 groups of 4 animals each. Five clearance T ½ values were determined on each animal with a 2- to 3-day interval between consecutive determinations.

Methods. The method used for determining the plasma half time clearance of ICG was essentially the same as that reported by Bonasch *et al*(4). The dye was prepared to a concentration of 2.5 mg/ml and adminis-

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