Such partial occlusion of the left renal artery was followed by cardiac hypertrophy (average +28%) in 9 rats. When there was simultaneous left nephrectomy there was of course no hypertrophy (+2%) in 14 rats.

Partial occlusion of the aorta between the kidneys resulted in cardiac hypertrophy in 7 rats (+26%); but when left (distal) nephrectomy was done simultaneously, cardiac hypertrophy failed to occur (—2%) in any of 25 rats. In some cases the occlusion had become complete, assurance that the ligature was sufficiently tight.

Finally, partial occlusion of the aorta above both kidneys resulted in cardiac hypertrophy (+20%) in 12 rats. This situation is analogous to that existing in coarctation of the aorta.

Conclusions. Partial (or even complete) occlusion of the aorta in rats produces hypertension only if there is living renal tissue distal to the occlusion, just as there must be a kidney beyond a partially occluded renal artery in order to produce hypertension in a Goldblatt dog. The same degree of mechanical obstruction due to stenosis of the aorta and the presence of a collateral bed never results in hypertension when all of the renal tissue is above the site of occlusion.

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Study of Some Variables Affecting the Prothrombin Time.*

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Of the 4 substances in the plasma (calcium, fibrinogen, thromboplastin and prothrombin) only 2, calcium and fibrinogen, can be estimated quantitatively. Since thromboplastin is derived presumably from platelets, estimations of the number and fragility of platelets are considered to be adequate tests for determining the presence of sufficient thromboplastic substance. Although prothrombin can be isolated from the blood in relatively pure form, the methods for its isolation are not adaptable to a study of fluctuations under pathologic and experimental conditions. Therefore, in determining the amount of prothrombin in a sample, it has been necessary to use methods of bioassay which depend upon a

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comparison of coagulation times. Howell[1] uses the coagulation time of recalcified plasma as the index of the amount of prothrombin present. This method may be criticized because it records variations not only in prothrombin but also in thromboplastin. The method of Quick, *et al.*,[2] by addition of an excess of thromboplastic substance, presumably leaves the prothrombin concentration as the only variable in the reaction.

However, other variables must be considered. Warner, *et al.*,[3] recognized the variations in the Quick reaction caused by substances other than prothrombin. In their prothrombin test, a globulin fraction of the plasma, presumably containing almost all of the prothrombin, is precipitated from the plasma, redissolved in oxalated saline, dialyzed, and then tested in a constant mixture of calcium-thromboplastin-fibrinogen. This method probably eliminates substances other than prothrombin which might influence the reaction. It should be noted, however, that substances in combination with plasma proteins that are incapable of removal by dialysis may be precipitated in the globulin fraction and may still influence the reaction. Our experiments show that the addition of various substances alters the prothrombin time as measured by the method of Quick.

The method of Quick,[2] with minor variations, was employed. Nine cc. of venous blood were mixed with 1 cc. of M/10 sodium oxalate, and centrifugalized; and the supernatant plasma was removed. One-tenth cc. of this plasma was mixed with 0.1 cc. of M/40 $CaCl_2$ and 0.1 cc. of fresh rabbit brain emulsion in a 13 mm. tube at 37°C.; and the time of coagulation was recorded with a stopwatch. In the control tests, 0.1 cc. of 0.85% NaCl was substituted for the brain emulsion. The emulsified brain was centrifugalized. This undiluted material represented a 100% emulsion, and from it serial dilutions to 0.001% were made. Progressively increasing concentrations were used to demonstrate any effect of an excess of thromboplastic material. Using the supernatant saline solution from similar emulsions up to a 20% concentration, Quick[4] demonstrated that the coagulation time could not be shortened beyond a minimum which was reached with a 10% emulsion. Each of Tables I to VII presents the results obtained in one of a series

[1] Howell, W. H., *Arch. Int. Med.*, 1914, **13**, 76.

[2] Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J Med. Sci.*, 1935, **190**, 501.

[3] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667

[4] Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

of experiments. In each table the figures shown were characteristic of all experiments of the series.

Table I demonstrates the apparent anticoagulant action of high concentrations of thromboplastic material.

TABLE I.
Effect of Progressively Increasing Concentrations of Rabbit Brain Emulsion on Coagulation Time of Normal Recalcified Plasma.
(Results in one of 15 experiments.)

% Brain Emulsion	100	75	50	25	20	15	10	5	4	3	2	1	½	.1	.01	.005	.001	0.85% NaCl Control
Coag. Time in Seconds	40	30	*25*	*23*	*23*	*24*	*23*	*24*	*23*	*23*	*22*	*25*	*26*	40	63	73	74	120

Table II shows that the inhibition of coagulation in high concentrate is not due to an excess of thromboplastin but is rather a physical characteristic of the reaction.

TABLE II.
Effect of Progressively Increasing Concentrations of Rabbit Brain Emulsion on Coagulation Time of Diluted* Normal Recalcified Plasma.
(Results in one of 5 experiments.)

% Brain Emulsion	100	75	50	25	20	15	10	5	4	3	2	1	½	.1	.01	.005	.001	0.85% NaCl Control
Coag. Time in Seconds	900	240	90	65	55	48	44	*38*	*37*	*37*	*38*	*42*	45	68	85	97	124	138

* A mixture of 40% plasma and 60% of a solution of 0.85% NaCl containing the same concentration of sodium oxalate as the original plasma.

If the prolonged coagulation time in high concentrations of brain emulsion were due to an excess of thromboplastin, there would be a gradual shift of the optimum concentration of brain emulsion toward the lower concentrations with progressive dilution of the plasma. It is apparent this is not true. The optimum concentration of rabbit brain emulsion was found to be between 0.5% and 50% for normal plasma. This optimum range was markedly narrowed by the use of any plasma exhibiting a prolonged coagulation time. Table II demonstrates narrowing of the optimum range with dilution of the plasma, and Table III narrowing of the range in icteric plasma.

A 5% rabbit brain emulsion supplied the optimum concentration of thromboplastic material in all conditions studied. In no instance was the rabbit brain perfused. While this omission may cause a slight prolongation of the coagulation time, it allows comparison with thromboplastic material from sources in which perfusion is not possible.

TABLE III.
Effect of Progressively Increasing Concentrations of Rabbit Brain Emulsion on Coagulation Time of Icteric Recalcified Plasma.
(Results in one of 15 experiments.*)

% Brain Emulsion	100	75	50	25	20	15	10	5	4	3	2	1	½	.1	.01	.005	.001	0.85% NaCl Control
Coag. Time in Seconds	600	150	120	55	51	44	*39*	*35*	*36*	*35*	*38*	*40*	45	54	78	104	125	180

*The coagulation time was similarly prolonged in all cases studied. The degree of prolongation varied with each individual case.

In all experiments, the oxalated blood was centrifugalized at 1800 r.p.m. for 10 minutes, and the supernatant plasma removed. There was no significant change in coagulation time whether high or low speed centrifugalization was used.

To test the effect of added substances on the coagulation time, the following method was employed: Equal quantities of a M/20 $CaCl_2$ solution and a solution of the substance to be tested, made up in twice the final concentration desired, are mixed. This produces a solution in which the $CaCl_2$ concentration is M/40, and the substance to be tested is of the desired concentration. Since in the performance of the test the $CaCl_2$ solution and the plasma are present in equal volume, any effect noted after the addition to the $CaCl_2$ solution of a foreign substance may be interpreted as if the substance were added directly to the plasma at the time of recalcification.

The coagulation time of normal plasma by this method is 22 to 25 seconds (Table I); that of icteric or diluted plasma may be prolonged to 70 seconds. Table II shows prolongation of the coagulation time in normal plasma diluted to 40%, and Table III in a case of obstructive jaundice (icterus index 110).

Table IV demonstrates progressive prolongation of the coagulation time after the addition of increasing amounts of heparin.

TABLE IV.
Effects of Progressively Increasing Concentrations of Heparin* on Coagulation Time of Normal Plasma in the Presence of an Optimum Concentration of Rabbit Brain Emulsion.
(Results in one of 4 experiments.)

Units of Heparin per cc. plasma	Normal control	0.1	0.2	0.3	0.5	1	2	3	4
Coagulation time in seconds	22	22	23	26	29	37	52	110	140

*Connaught Laboratories—1000 units per cc.

As is shown in Table V, increasing quantities of NaCl caused a progressive prolongation of the coagulation time even in the pres-

ence of an optimum concentration of all of the elements necessary for coagulation of the plasma.

TABLE V.

Effect of Progressively Increasing Concentrations of NaCl on Coagulation Time of Normal Plasma in the Presence of an Optimum Concentration of Rabbit Brain Emulsion.

(Results in one of 4 experiments.)

NaCl added	Control	.10N	.20N	.30N	.40N	.50N	.60N	.70N	.80N	.90N
Coagulation time in seconds	22	24	27	30	33	37	42	52	65	72

Quick,[5] using a thrombin-fibrinogen mixture, demonstrated that coagulation is definitely altered by the presence of electrolytes. That there is a qualitative difference between various simple salts, independent of the total concentration of electrolytes, is demonstrated in Table VI.

TABLE VI.

Effect of Increasing Progressively the Concentrations of $CaCl_2$ on the Coagulation Time of Normal and Icteric Plasma in the Presence of an Optimum Concentration of Rabbit Brain Emulsion, the Total Electrocyte Content Being Held Constant.*

(Results in one of 4 experiments.)

% CaCl	.05	.10	.15	.20	.25	.30	.35	.40	.50	.60	.70	.80	.90	1.00
Icteric Plasma Time in Seconds	74	*44*	*41*	*41*	*42*	*42*	48	53	62	70	84	87	93	122
Normal Plasma Time in Seconds	65	*30*	*32*	*30*	*32*	*35*	37	39	42	47	55	60	65	67

*Solutions made by mixing 0.35N $CaCl_2$ (1.925%) and 0.35N NaCl (1.225%) in varying quantities to attain the desired calcium concentration.

In this instance the concentration of Cl ion remains constant while the quantities of Na and Ca ion are reciprocally varied. Even though the total electrolytes are held constant, there is a progressive lengthening of the coagulation time with increasing quantities of Ca ion. The optimum concentration of Ca for recalcification of oxalated plasma is the same in normal as it is in icteric blood.

The effect of the addition of complex salts to the plasma is more striking (Table VII).

The addition of 400 mg. % ox bile salts (sodium taurocholate and sodium glycocholate) to normal plasma produces the same alteration in coagulation time as is found in icteric plasma. While this concentration of bile salts is above that usually stated to be present in the blood of patients with obstructive jaundice (6 to 20 mg. %), it must be remembered that no accurate quantitative

[5] Quick, A. J., *Am. J. Physiol.*, 1936, **115**, 317.

TABLE VII.

Effect of Progressively Increasing Concentrations of Ox Bile Salts* on Coagulation Time of Normal Plasma in the Presence of an Optimum Concentration of Rabbit Brain Emulsion.
(Results in one of 3 experiments.)

Concentration of bile salts, mg. %	50	100	150	200	250	300	400	500	600	700
Coagulation Time in Seconds	22	23	24	26	28	30	35	40	65	no clot

*Fairchild Bros. & Foster.

methods are available for the isolation of bile salts from icteric blood.

Prolongation of the coagulation time was observed also in one case of uremia (blood N.P.N. 238 mg. %). The coagulation time was found to be 32 seconds, as compared with 22 seconds for the control.

Table VIII is a summary of the findings given above.

TABLE VIII.

Comparison of Coagulation Times of Plasma in Presence of an Optimum Concentration of Thromboplastic Substance in Various Experimental and Pathologic Conditions.

Experiment	Coagulation Time, Seconds	Probable Mechanism of Action
Normal	22	Increased activation of prothrombin by thromboplastin
Diluted plasma (1)	37	Diminution of prothrombin
Heparinized plasma (2)	37	Antithrombic action of heparin
Plasma with added NaCl (3)	33	Interference with fibrin formation
Uremic plasma (4)	32	Mechanism not proven
Icteric " (5)	35	
Plasma with added $CaCl_2$ (6)	35	
Plasma with added ox bile salts (7)	37	

(1) 40% plasma and 60% 0.85% NaCl.
(2) 1 unit Heparin (Connaught Lab.) per cc. plasma.
(3) 1.7% NaCl added.
(4) N.P.N. 238 mg.%.
(5) Icterus index 110.
(6) 0.35% $CaCl_2$ in 1.2% NaCl. Total normality of chlorides 0.35.
(7) 400 mg.% ox bile salts (Fairchild Bros. & Foster).

Summary. The addition of various substances to normal plasma caused a prolongation of the coagulation time as measured by the Quick reaction. Therefore prolonged coagulation time by this method does not always indicate a deficiency of prothrombin in the plasma.