still present after bilateral vagotomy and denervation of the carotid sinuses. 3. The effects of variations of blood pressure are not restricted to the visceral nervous system but extend to the somatic nervous system, inasmuch as increase in blood pressure is associated with decreased somatic excitability and decrease in pressure is associated with increased somatic excitability.

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Production of "Prothrombin Deficiency" and Response to Vitamins A, D and K.

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This investigation was undertaken because it was found that rats receiving a diet containing 20% of mineral oil by weight developed a hemorrhagic tendency. On examining their "prothrombin time" it was found to be prolonged. It was thought that the large doses of mineral oil prevented the absorption of vitamin K as well as vitamin A and carotene.

Normal Prothrombin Time in Rats. Methods. The control diet consisted of Purina Dog Checkers and water *ad libitum*. The determination of the prothrombin time was made by a modification of Quick's[1] method. Two cc of blood obtained by cardiac puncture (22 gauge needle) under light anesthesia is drawn into 0.2 cc of 1.34% sodium oxalate solution. This is diluted to 4.0 cc with 0.9% NaCl solution and centrifuged. 0.1 cc of the diluted oxalated plasma is mixed with 0.1 cc of thromboplastin solution. 0.1 cc of 1.11% calcium chloride (anhydrous) solution is quickly added and the time for clot formation is recorded with a stop-watch. The thromboplastin solution was made by weighing, macerating and spreading fresh rat's brain on gauze so that 1 sq in. of gauze contains 0.5 g of fresh brain. This is dried and preserved at room temperature in a desiccator. When it is to be used, one of the squares is leached in 5 cc of saline solution (0.9%), and the solution incubated at 54°C for 10 min. The supernatant fluid is used. A fresh solution is made and checked for activity daily. The dried-brain-gauze preparation maintains its activity for several weeks. Chemically clean glassware was

[1] Quick, A. J., *J. Am. Med. Assn.*, 1938, **110**, 1658.

TABLE I.
Frequency Distribution of Prothrombin Time of Normal Rats.

Interval time in sec.	43-46	47.50	51-54	55-58	59-62	63-66	67-70
No. of cases	2	2	3	6	15	17	19
Interval time in sec.	**71-74**	**75-78**	**79-82**	**83-86**	**87-90**	**91-102**	**103-106**
No. of cases	**18**	**15**	**14**	**6**	**2**	**0**	**1**

used, and 3 determinations on each sample of blood were made and averaged. The clotting time was recorded at the first sign of formation of a coagulum. Blood samples showing hemolysis were discarded.

Results. 120 rats, weighing 200-370 g and receiving the control diet from weaning, were selected at random from our colony. The results are shown in Table I. The mean prothrombin time was 69.8 ± 0.9 (S.E.) seconds. The standard deviation of the distribution was 9.8 seconds. No significant sex difference was obtained. Of 190 cardiac punctures performed on normal rats, 12.6% proved fatal.

Effect of Repeated Cardiac Puncture. The effect of repeated hemorrhage had to be controlled in order to interpret the subsequent results. Thirty animals were placed on the control diet and their prothrombin time was determined weekly for 4 weeks. The initial average prothrombin time was 70.2 seconds, range, 43-103; 1st week, avg 69.2, range 44-93; 2nd week, avg 65.4, range 35-100; 3rd week, avg 70.1, range 59-85; 4th week, avg 71.4, range 55-86. The fourth week includes only 17 of the rats. Six of the rats were studied for 13 weeks; average 68 sec, range 61-79. The results showed that the prothrombin time of any given rat was reliable from week to week within normal limits and that no correlation exists from week to week in the rank order of the prothrombin time of individual rats. Thus weekly bleeding did not significantly affect the prothrombin time.

Effect of Mineral Oil with Agar. Method. Sixty-six normal rats, weighing 200-370 g, were placed on a diet consisting of 7 parts by weight of the powdered control diet, mixed with 3 parts of "Petrol-agar". This diet contained 20% by weight of mineral oil. *Results.* The results are shown in Table II. Fifty-three of the 66 rats, or 85%, developed a prolongation of the prothrombin time within 36 days after being placed on the diet. Two of the remaining 13 did not develop a deficiency during 77 days' observation. Of the remaining 11, 4 died as a result of cardiac puncture prior to 30 days; 5 were not studied longer than 22 days, and 2 not longer than 30 days. The average control prothrombin time of the group of 66 rats was 70 seconds, range 46-90 seconds. The median prothrombin time of the

TABLE II.

Showing Effect of a 20% Mineral Oil (30% Petrolagar) in the Diet on the Prothrombin Time in Rats.

Rat No.	1	2	3	4	5	6	7	8	9	10	11
Normal P.T. in sec	80	66	66	85	85	68	80	77	60	65	66
Days on diet	22	22	22	24	24	24	21	21	28	21	21
P.T. after diet	1800+	1800+	166	204	271	485	450	103	86	1800+	1800+
Rat No.	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Normal P.T. in sec.	66	80	74	80	73	75	79	90	70	84	71
Days on diet	23	23	20	20	16	16	16	17	17	28	28
P.T. after diet	660	1800+	1800+	600	480	1800+	780	223	286	538	201
Rat No.	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
Normal P.T. in sec	52	64	77	68	65	71	54	67	88	46	67
Days on diet	28	28	27	27	27	77	77	27	20	30	30
P.T. after diet	209	260	241	466	393	88	82	217	138	76	70
Rat No.	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**
Normal P.T. in sec	67	56	82	84	73	58	67	56	61	81	68
Days on diet	22	22	30	30	30	22	22	21	21	21	21
P.T. after diet	212	67	229	209	233	192	159	145	189	133	147
Rat No.	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**
Normal P.T. in sec	70	75	70	80	74	77	64	73	66	71	68
Days on diet	21	30	30	36	36	20	20	20	20	20	20
P.T. after diet	143	138	205	197	190	162	149	70	62	225	239
Rat No.	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**66**
Normal P.T. in sec	76	62	86	60	82	65	55	48	64	68	54
Days on diet	20	20	22	22	35	21	35	20	22	22	30
P.T. after diet	149	158	245	170	129	146	154	137	93	78	100

"P.T." = Prothrombin time in seconds.

The normal average prothrombin time was 70±1.16 (S.E.) sec; range 46-90 sec.

The prothrombin time after an average of 25.4 days on the diet was 199 sec.

56 rats proven to have a deficiency between the 16th to 36th days on the diet was 199, range 118-1800+. In 7 of the 56 the prothrombin time was more than 30 minutes, which explains why the median rather than the mean of this group was used. No significant changes in weight occurred. Most of the rats remained at about their initial weight.

Effect of Mineral Oil Alone. Method. Five normal rats, weighing 200-275 g were placed on a diet consisting of 8 parts of the control diet and 2 parts of mineral oil. *Results.* The average prothrombin time on the control diet was 63.4, the range 49-74 seconds. All 5 animals developed a prothrombin deficiency within 30 days, the average prothrombin time being 365.8, the range 118-586 seconds. This experiment was done to determine whether the mineral oil in the "Petrolagar" was alone concerned.

Effect of Returning Rats with a Prothrombin Deficiency to the Control Diet. Method. A prothrombin deficiency was produced in 11 rats by the "Petrolagar diet". Then they were returned to the control diet. *Results.* The average prothrombin time of the 11 rats before they were given the "Petrolagar diet" was 77.1 seconds, range 66-90. After an average of 21 days on the diet the median prothrombin time was 480 seconds, range 103-1800+. On returning the rats to the control diet, the prothrombin time decreased to an average of 66 seconds after an average of 8.6 days.

Effect of Vitamins A, D and K Parenterally on Correcting the Prothrombin Deficiency. Method. Thirty-three rats were selected after their prothrombin time had been increased by the "Petrolagar diet". While this diet was being continued, 10 were given 50,000 I.U. of Vitamin A in oil subcutaneously by a single injection; 11 were given 500 I.U. of Viosterol in oil subcutaneously; and 8 were given 2,000 Almquist units of Vitamin K subcutaneously. A week later the prothrombin time of the rats was again determined. *Results.* The results are shown in Table III. The Vitamin A as administered had no effect. The Viosterol caused a significant improvement. The Vitamin K returned the prothrombin time to normal. Oil free of vitamin was not used because the oil containing Vitamin A caused no improvement and served as a control group for oil alone.

Discussion. It has been observed by us and others[2] that the administration of mineral oil in large amounts (20% by weight in the diet) will in time produce the manifestations of a Vitamin A deficiency. The failure of Vitamin A to return the prothrombin time

[2] Isaacs and Ivy, unpublished data; Dutcher, Harris, Hartzler and Guerrant, *J. Nutr.*, 1934, **8**, 269.

TABLE III.
Showing the Effect of Vitamins A, D and K on Prothrombin Deficiency in Rats.

Vitamin subcut.	No. of rats	Prothrombin time on control diet in sec		Prothrombin time on Petrolagar diet in sec		Prothrombin time 1 wk after vitamins	
		Avg	Range	Avg	Range	Avg	Range
A, 500,000 I.U.	10	70	61-81	168	132-225	202	90-340
D, 500 I.U.	11	70	48-82	182	145-235	121	70-140
K, 2,000*	8	69	52-86	279	185-590	70	55-97

* The Vitamin K is expressed in Almquist Units and was a concentrated preparation obtained from Abbott Laboratories. The Vitamin D was an activated ergosterol concentrate in oil obtained from Meade, Johnson and Co. The Vitamin A was a concentrate, 100,000 I.U. per cc, in oil obtained from Abbott Laboratories. Vitamin K-free oil was used as a diluent.

to normal indicates that the prothrombin deficiency was not due to a Vitamin A deficiency. Further work is required to show whether a deficiency of Vitamin A plays any rôle. Vitamin D was used because it has been observed to be of value for the hemorrhagic tendency in jaundiced patients.[3] How Vitamin D acts to decrease the prothrombin time is conjectural. This vitamin has been observed to cause a thrombocytosis and a shortening of the coagulation time in rats.[4] We have not made a study of the thrombocytes. The response to Vitamin K was to be anticipated. From our data it cannot be concluded whether the mineral oil produced the prothrombin deficiency by preventing the absorption of Vitamin K or by producing hepatic injury; but the latter possibility is unlikely. This method of producing a prothrombin deficiency is very convenient and has potentialities for being developed into an assay method for Vitamin K.

Summary. 1. Prothrombin deficiency has been produced in rats by feeding an adequate diet containing 20% by weight of mineral oil. 2. The subcutaneous administration of Vitamin K corrected the prothrombin deficiency; activated ergosterol definitely improved the deficiency; Vitamin A had no effect on the deficiency.

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Changes in Arterial Pressure after Bilateral Complete or Partial Ureteral Occlusion.*

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Recently, we have been investigating the effect of hydronephrosis on the arterial blood pressure. The blood pressure determinations in this study were made with the Hamilton manometer[1] on trained unanesthetized dogs according to a technic previously described by us.[2] In addition the blood non-protein nitrogen was determined before and after operation.

In 7 dogs complete bilateral ureteral occlusion was performed

[3] Gray and Ivy, *Am. J. Digest. Dis.*, 1935, **2**, 368; McNealy, Shapiro and Melnick, *Surg. Gyn. and Obst.*, 1935, **60**, 785.

[4] Phillips, Robertson, Corson and Irwin, *Ann. Int. Med.*, 1931, **4**, 1134; Tocantius, *Medicine*, 1938, **17**, 155.

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[1] Hamilton, W. F., Brewer, J., and Brotman, I., *Am. J. Physiol.*, 1934, **107**, 427.

[2] Katz, L. N., Friedman, M., Rodbard, S., and Weinstein, W., *Am. Heart J.*, 1939, **17**, 334.