8. Schwentker, F. F., and Comploier, F. C., *J. Exp. Med.*, 1939, v70, 223.
9. Cavelti, P. A., and Cavelti, F. S., *Arch. Path.*, 1945, v39, 148.
10. Kay, C. F., *Am. J. Med. Sc.*, 1942, v204, 483.
11. Farr, L. E., and Smadel, J. E., *J. Exp. Med*, 1939, v70, 615.
12. Hawn, C. v. Z., and Janeway, C. A., *J. Exp Med.*, 1947, v85, 571.
13. More, R. H., and Waugh, D., *J. Exp. Med.*, 1949, v89, 541.
14. Waugh, D., and More, R. H., *J. Exp. Med.*, in press.
15. Fahr, Th., in *Henke-Lubarsch, Handb. d spez. path. Anat. u. Histol.*, 1925, VI/i, 290, Berlin.
16. Ellis, A. W. M., *Lancet*, 1942, vI, 1, 34, 72.
17. Schoenheimer, R., Ratner, S., Rittenberg, D., and Heidelberger, M., *J. Biol. Chem.*, 1942, v144, 541, 546, 555.
18. Foord, A. C., *J. Infect. Dis.*, 1918, v23, 159.
19. Graziani, A., *Centralbl. f. Bakteriol.*, 1, 0., 1908, v42, 633.

Received February 1, 1952. P.S.E.B.M., 1952, v79.

Experiments with C^{14}-Menadione (Vitamin K_3)* (19459)

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Dam(1) established that hemorrhage in chickens on certain diets was due to the absence of a new accessory food factor, vit. K. Schonheyder(2) established that the hemorrhagic condition was due to a decrease in plasma prothrombin, and Greaves(3), following up the work of Hawkins and Brinkhous (4), established that the prothrombin deficiency in obstructive jaundice was due to failure to absorb vit. K, the presence of bile in the intestine being necessary for this. Extensive experimental and clinical observations (5-7) have been made connecting a decrease in plasma prothrombin concentration with interference with liver function. By inference, it has been assumed that vit. K acts through the liver, although little evidence has been obtained for this. Likewise little information is available regarding distribution of the vitamin in tissues. The senior authors have previously reported the results of studies of dicumarol labelled with C^{14}(8). As this substance and vit. K appear to be related as antivitamin and vitamin, radioactive K has been synthesized as 2:C^{14}-methyl-1:4-naphthoquinone(radioactive menadione) and administered to mice and dogs. The present communication reports the results of these experiments.

Methods. Experimental procedure. 2-methyl-1:4-naphthoquinone with C^{14} in the methyl group was synthesized by Mr. R. V. Phillips, as described elsewhere(9). The specific activity was 4.10 x 10^4 counts per minute per mg (c.p.m./mg). The material was dissolved in corn oil and injected intramuscularly into dogs and mice. The mice were sacrificed at different time intervals and the activity determined on aliquots of the dried tissues. To determine the activity of the dog tissues, the organs were boiled in dilute alkali and an aliquot counted. Radioactivity measurements were made using a gas-flow type of Geiger-Müller Counter. The efficiency of the counting system was checked frequently with a sample of C^{14}-dicumarol used as a standard. Counts were corrected for background, efficiency change with time, self-absorption, resolving time and geometry. Twice the standard deviation of the background was 9.6

TABLE I. Test of Radioactivity of Expired CO_2 of Mouse after Receiving C^{14}-Menadione.

Sample hr	Wt of $BaCO_3$ counted, mg	Observed activity −bkgd. c.p.m.	Total activity
0-11	11	2.6	N.A.
11-17	36	7	
17-24	60	3.8	

N.A. = No significant activity.

* Assisted by grants from the National Research Council of Canada to L. B. Jaques and J. W. T. Spinks.

c.p.m. and a count greater than background by this amount was considered significant. Values differing from background by less than this are reported as no activity (N.A.). A depletion of vit. K in dogs was effected by a cholecystonephrostomy according to the Kapsinow-Markowitz(10,11) technic, anastamosing the gall bladder to the right kidney. A total of 6 dogs were prepared in this way. A weekly check was made for the detection of bile in the feces and urine to ensure that the cholecystonephrostomy was complete. After death. the animal was checked at autopsy to make sure that the fistula was patent and that there were no other channels for bile. It took from one to 3 months after the cholecystonephrostomy for the prothrombin time to increase to double its normal value. Prothrombin times were determined by the usual method using 0.02 M calcium chloride solution and a commercial rabbit-brain powder supplied by Cappel Laboratories. Wayne. Pa.

Experiments with normal mice. Expired CO_2. To check the possibility of the radioactive methyl group being metabolized to CO_2. a 27 g mouse was injected with 1.0 mg of vit. K_3, placed in a metabolism cage for 24 hours and the expired CO_2 collected from the mouse. The results are shown in Table I.

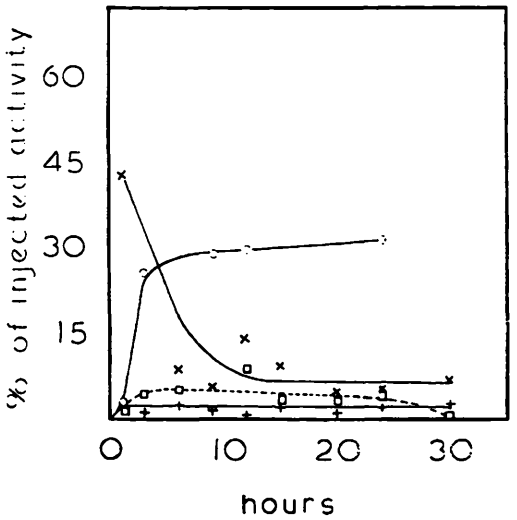

FIG. 1. Radioactivity in mice after inj. of C^{14}-vit. K_3. 1 mg/mouse intramusc. into right hind-leg. ×——× inj. site; ○——○ urine; □——□ gastrointestinal tract and contents; +——+ blood.

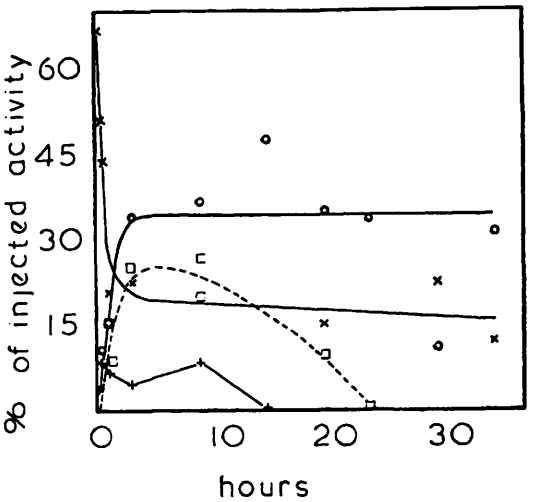

FIG. 2. Radioactivity in mice after inj. of C^{14}-vit. K_3. .1 mg/mouse intramusc. into right hind-leg. ×——× inj. site; ○——○ urine; □——□ gastrointestinal tract and contents; +——+ blood.

It can be seen that there is no activity in the expired CO_2 from a mouse, following C^{14}-vit. K_3 administration.

Tissues. Two series of mice were injected with the labelled vitamin and then sacrificed in succession at varying times. Twelve mice received 0.1 mg of vit. K and eleven received 1.0 mg of the vitamin. Excreta were collected up to the time of sacrifice at which time, tissues and organs were removed. All samples were tested for radioactivity. The results are shown for blood and excreta in Fig. 1 and 2 and for tissues in Table II. The vitamin was injected intramuscularly into the right hindleg and the latter was counted separately and reported as the activity of the injection site. In the case of the zero sample, the animal was sacrificed and the K then injected. 94% of the activity was recovered from the injection site of this animal. It can be seen that the activity disappeared rapidly from the injection site in the first hour after the injection, leaving a small amount of residual activity in the depot. This rapid loss of activity from the injection site was accompanied by the appearance of activity in the blood. The total amount of activity in the blood was calculated on the assumption that the weight of the total blood volume was 10% of the body weight. With the larger dose, the ac-

TABLE II. Radioactivity of Tissue of Mice after Injection of C^{14}-Menadione.

Time after inj.	Liver		Lung		Kidney		Spleen		Muscle		Carcass	
	Dose, mg											
	.1	1	.1	1	.1	1	.1	1	.1	1	.1	1
0	*	—	*	—	*	—	*	—	—	—	*	—
5 min	"	.42	"	.03	"	.34	"	—	—	—	"	—
15	"	*	"	.06	.88	.56	"	—	—	—	"	—
30	"	"	.58	*	.83	*	"	—	—	—	"	—
					.85							
60	"	.23	"	—	.75	.34	"	—	—	*	"	2.24
		.34	1.17	—	*	2.34	—	*	—	"	—	*
3 hr	"	*	.49	—	"	.17	*	"	—	"	*	"
			*									
6	—	.60	—	—	—	.26	—	.04	—	4.48	—	5.72
9	*	.45	*	—	*	*	*	*	—	*	*	*
			.21									
12	—	1.71	—	—	—	.49	—	"	—	"	—	"
15	*	.32	.36	—	*	.15	*	"	—	"	*	2.75
20	"	*	*	*	"	.66	"	"	—	—	"	*
24	"	"	"	.04	"	.30	.85	"	—	—	"	"
30	"	"	"	*	—	*	*	"	—	—	"	"

All values given as % of injected dose; 1 mg of C^{14}-menadione $= 4.10 \times 10^6$ counts/min. Mice 18-26 g weight.

* No significant activity (<9.6 c.p.m./sample).

tivity per mg of dry sample and consequently the total blood activity was approximately twice that with the smaller dose, and the activity could be detected for a longer period of time. 0.3% of the larger dose, and 3% of the smaller, distributed in the mouse total blood volume would be detectable. Relatively little activity was found in tissues. Liver, lung and kidney occasionally showed small amounts of activity. However, the values were just within the limits of significance and accounted for only 1% of the activity injected. Skin, bone and muscle of some mice were treated separately with no significant activity showing. The results are included under carcass. The activity in the kidney may be explained as due to activity in glomerular filtrate and urine, since values of the same order were obtained on urine taken directly from the bladder. Activity in lung was observed in an irregular manner, in liver only with the 1 mg dose. In the latter case for the eight mice sacrificed between 0 and 12 hours, six had livers which gave counts more than 12.8 c.p.m. above background (significant at the 0.05 level) and the average activity in the liver was equivalent to 5 μg of vit. K. 30-40% of the activity injected was recovered in the urine. This excretion occurred largely in the first 3 hours after the injection. No activity was found in the feces. The gastrointestinal contents showed some activity reaching a peak 3 hours after the injection for the smaller dose. The gall bladder and contents showed traces of activity coincident with that in the gastrointestinal tract.

Blood. In view of the small residual amount of radioactivity which appeared to persist in blood, in a further experiment a fractionation of the blood was attempted, both to concentrate the activity and thus get a more accurate count, and to determine the fraction of the blood containing the activity. The fractionation procedure followed was that of Cohn *et al.* (12). Fourteen mice were injected with a total of 12 mg of vit. K_3. These were sacrificed at the end of 15 hours and the blood collected by cardiac puncture. A total of 6.0 cc of blood was obtained from the 14 animals. The blood was drawn with a silicone-coated syringe and placed in a silicone-coated graduated centrifuge tube where it was diluted with 0.5 cc of 3.8% sodium citrate solution. The blood was immediately transferred to the cold room at −5°C for fractionation. After removal of 0.5 cc of blood for counting, the remainder was centrifuged at high speed. The plasma and white cells were drawn off separately and the red cells washed twice with 2.0 cc of isotonic saline. The wash from the red cells was

TABLE III. Radioactivity of Various Fractions from Pooled Blood of 14 Mice 15 Hr after Injection of C^{14}-Vit. K.

Fraction	Observed activity (minus background) c.p.m.	Total activity, c.p.m. × 10^3	% total injected activity
Whole blood	10	1.40	.29
Hemoglobin	11	1.15	.23
Washed r.b.c.	11	1.05	.21
W.b.c. and platelets	9.6	.051	.01
Wash from r.b.c. and platelets	33	.282	.06
Ghosts	5.1		*
Albumin, V.	22	.244	.05
R-globulin, II	4.5		*
Prothrombin, III-1,2	2.6		"
Fibrinogen, I. III-3	5.1		"
Cholesterol, III-0	1.9		"
Cholinesterase, IV	5.1		"
Small albumins, VI	1.9	.109	.02

* No significant activity.

added to the platelets and w.b.c. and centrifuged. The plasma was poured into a 50 ml plastic centrifuge tube and precipitated with ethanol, the fractions being prepared as described by Cohn *et al.* The counts on the various fractions are shown in Table III. Significant activity was found in whole blood, hemoglobin, washed red blood corpuscles, white cells and platelets, the wash from the r.b.c.'s and platetlets, and the albumin-fraction. The albumin (fraction V and VI) and the wash (from r.b.c.'s platelets) alone showed a sufficiently high-specific activity to give reliable estimates of activity. With the relatively gross errors in the estimations of activity on whole blood and hemoglobin, due to low specific activity, it is difficult to estimate how much radioactivity is associated with the latter but in as much as the wash from the r.b.c.'s had a higher specific activity than the albumin fractions and an equal total activity, this indicates that the r.b.c.'s showed a higher total activity than the albumins. There was definitely no activity associated with the fibrinogen, prothrombin or globulin fractions.

Urinary products. In order to examine the nature of the radioactive urinary products, urine was subjected to paper chromatography. Fresh urine was used from a mouse which was injected with 2 mg of vit. K_3. Two drops of this urine placed on a one inch square of filter paper registered 1.92 x 10^3 c.p.m. The solvent used was made up of 3 parts butanol to 1 part methanol and saturated with water, to which was added a few drops of acetic acid. Two drops of the urine was placed on one end of a filter paper strip one inch in width. These strips were suspended in beakers containing the solvent mixture and were developed for 30 hours inside a large covered glass cylinder. At the end of 30 hours the strips were dried and cut in 1 cm lengths for counting. Fig. 3 shows the location of the radioactivity on the strip. A radioactive component with an R_F value of 0.41 is present. Two drops of 2 N acetic acid were added to 4 drops of urine and the sample was subjected to hydrolysis by heating at 100°C for 2 minutes. When a chromatogram was developed in the same way a second component ($R_F = 0.25$) was observed along with a decrease in the amount of the first component. This second component has also been obtained consistently with urine that has stood some days, suggesting that this compound can be formed also by bacterial action.

Experiments on cholecystonephrostomized dogs. Vit. K-deficient dogs were produced by performing a cholecystonephrostomy one to 3 months prior to the experiment. At the time of the experiment, the animals had a prothrombin time of 11-20 seconds (normal—8.2 seconds) and all showed a very pronounced

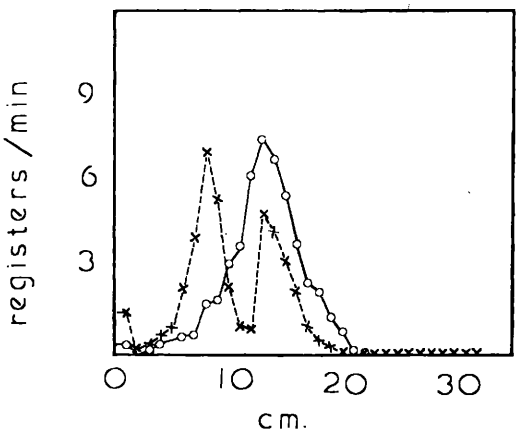

FIG. 3. Location of radioactivity on chromatograms of urine after inj. of C^{14}-vit. K_3. Mouse received 2 mg of C^{14}-vit. K intramusc. into right hind-leg. ○——○ fresh urine; ×——× urine after heating in .5 N acetic acid. 1 register = 64 counts.

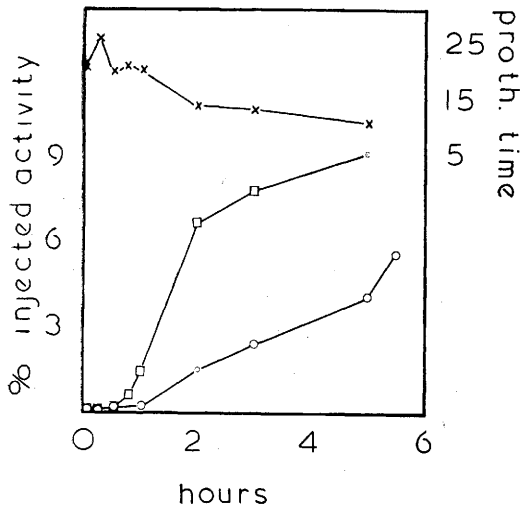

FIG. 4. Excretion of radioactivity and prothrombin time of cholecystnephrostomized dog. 1 mg/kg C^{14}-vit. K_3 inj. intramusc. at time zero. ×——× prothrombin time in sec.; □——□ urine from left kidney only; ○——○ bile.

clinical tendency to hemorrhage. The animals were anesthetized with nembutal, the left ureter cannulated to collect urine and the gallbladder to collect bile. Hemorrhage from this procedure occurred and was controlled with topical thrombin, but the animals died in 5 hours. One mg of radioactive vit. K per kg body weight was injected intramuscularly into the right hind-leg. Blood, urine, and bile samples were taken at intervals for determinations of radioactivity and aliquots of all tissues were taken at autopsy. The results of one experiment are shown in Fig. 4. The dog weighed 6.8 kg and had a prothrombin time of 19.4 seconds. 6.43 mg of C^{14}-vit. K_3 were injected intramuscularly into the right leg. Samples of blood, urine and bile were withdrawn simultaneously at 1, 5, 15, 30, 45 and 60 minutes, then at the 2-, 3- and 5-hour intervals after the injection. 0.2 ml of blood was used for counting each time. No activity was found in the blood. The prothrombin time and excretion of radioactivity in the urine and bile are shown in Fig. 4. The dog died 5 hours after the injection. On death, tissues were removed for measurement of radioactivity. The liver, blood, bone marrow, heart, spleen and kidneys showed no activity. The intestines with contents contained 16.4%, the site of injection 30.4% and the lungs 3.2%. Total activity of the urine samples collected from the left kidney was 9.0%, from the urinary bladder at the end of the experiment (right kidney) 5.0% and in the bile was 6.0% of the injected dose so that 70% of the activity injected was recovered. Urinary excretion of activity began thirty minutes after the injection and was at a maximum between the 1st and 2nd hour. The rate of excretion of activity in the urine in Fig. 4 is somewhat difficult to interpret as it must be noted that this is only for one kidney. Also there is the possibility that this kidney may be hyperactive with regard to urine secretion in order to compensate for the atrophied condition of the right kidney. However, the right kidney was found to be at least partially active as shown by the presence of activity in the urine collected from the bladder at the termination of the experiment.

In view of the smaller dose of labelled vit. K (1 mg/kg) given to the dog compared to the mouse, it would not be possible to detect activity in any tissues in this experiment unless a marked accumulation and concentration of activity had occurred. For instance, if the 2.6×10^5 c.p.m. were uniformly distributed in the total blood volume, there should be 388 c.p.m./ml of blood or 77 c.p.m. per sample counted. Hence it was necessary for over 10% of the injected dose to be present in the blood for it to be detected, and similarly 3% of the dose in the liver. However, with these limitations, no activity was present in the blood or liver. In contrast, a just significant amount of activity was present in the lungs. A second animal gave identical results except that no significant activity was found in the lungs.

Discussion. The highest dose, 1 mg per mouse, is as high as can be used safely if pharmacological effects are to be avoided. In a 20 g mouse this dose is equivalent to 50 mg/kg body weight which is the LD_{10} by the intraperitoneal route(14).

There is a very rapid absorption of the vitamin on intramuscular injection in both mice and dogs. One dog had no activity left at the site of injection within 5 hours, where-

as the other dog still had 30% present at the end of 5 hours. In the mice experiments it was found that 80% had been absorbed by the end of 4 hours. After the eighth hour there appeared to be very little further absorption, with activity still remaining after 35 hours. No pathological studies were made of the site of injection. However, Lord, Andrus and Moore(13) reported that no toxicity was evident at the site of intramuscular injections of menadione in corn oil in rats. The rapid rate of absorption of the vitamin from the site of injection in the mice is reflected in a rapid rise in blood activity. At each dosage level the amount of activity in the blood rose within 10 minutes to a maximal value of about 6.3 and 1.6% respectively and this level was maintained for hours.

A cholecystonephrostomy appeared to be effective in establishing a depletion of vit. K in dogs as evidenced by the very profuse hemorrhage on laparotomy. Depending upon individual variation between animals it took from one to 3 months to increase the prothrombin time to double its normal value. From the results reported it may be seen that the prothrombin time was back close to the normal value within 5 hours after the injection of radioactive vit. K. Andrus and Lord(15), working with rats, found that as little as 2 mg of menadione injected intramuscularly restored the plasma prothrombin by as much as 48% and the effect was evident as early as 8 hours after the injection. Garnett (16) found that a single intramuscular injection of vit. K in chicks with a prolonged blood coagulation time due to a diet deficient in vit. K, reduced the time to normal within one hour with a minimum effective dose of 0.135-0.27 mg in 3-week-old chicks weighing about 100 g.

Only in the mice receiving 1 mg (circa 40 mg/kg) was any activity found in the liver. An activity amounting to about 0.4% was present up to 15 hours after which time no further activity could be found. No activity was found in any of the dog livers. This observation shows quite definitely that the liver does not concentrate vit. K in contrast to dicumarol. Jaques and Spinks found that 20% of injected C^{14} dicumarol could be recovered from the liver. The activity of 10-20% of an equivalent injection of vit. K (0.25 mg) should have given 10^8-2 x 10^8 c.p.m. Actually, 1.0 mg of vit. K was administered and 20% (200 μg) would have an activity of 8.2 x 10^3 c.p.m. Canessa(7) gives values for the concentration in normal liver in different species equivalent to 20-250 μg of menadione per 100 g of tissue. A trace of activity was found in kidney and occasionally in lung and these gave actually higher specific activities than liver. The appearance of activity in the bile of the dogs indicates that a portion of the activity is excreted via this route. This is also indicated by the rapid appearance of activity in gall bladder and gastrointestinal tract in the mice. However, since it does not appear in the feces, this must be reabsorbed in the lower intestinal tract to explain the rapid fall in radioactivity of the gastrointestinal contents reported in Fig. 2.

From time to time, it has been postulated that vit. K serves as a prosthetic group of prothrombin, in which case the radioactive carbon should be recovered from prothrombin, after time has been allowed for synthesis to occur. However, the residual activity which appeared fixed in the blood was not attached to prothrombin. The albumin was the only fraction of the plasma proteins with which activity was associated. It had an activity of 0.07%. Fieser(17) found 75% of the vitamin in blood, bound to the albumin fraction. As shown in Table III, a larger quantity (0.23%) was found associated with the red cells. As no activity could be detected associated with the ghosts on hemolysis the activity must either be adsorbed on the red cells or combined with hemoglobin.

It was found in the mice experiments that there is a very rapid elimination of activity by the kidneys. Maximum excretion (totalling about 30%) is attained within 3 hours and no further increase was noted even after 24 hours. Urine samples from the bladder of 15-, 20- and 30-hour mice showed only 1% activity present and this is a further indication that after the first 3 hours, there is no further significant elimination by the kidneys. This maximal value of excreted activity of 30% corresponds with Richert's(18) observa-

tions on rabbits that 31-42% of a quinone was excreted in the urine after menadione administration, although he later reported that oxidative hydrolysis with ceric sulfate yielded quinone values 30% higher than by the previous hydrochloric acid procedure. Richert identified the product in the urine as 4-hydroxy-2-methyl-1-naphthyl sulfate. Doisy(19) has reported that when menadione is administered to rats 13-20% is excreted in the urine of which a part appears to be a β-glucuronide. Although no colorimetric analysis for the presence of a quinone was carried out, it may be gathered that it is excreted in a conjugated form. Richert found it necessary to hydrolyse the urine to obtain the color reaction and in the chromatographic analyses which were carried out on the active urine, the activity was found to undergo hydrolysis very readily giving rise to two compounds containing activity. Further, from the chromatographic work it was also seen that such hydrolysis would occur spontaneously due to bacterial action, so that evidence for the excretion of only one compound containing activity would be found only with fresh urine samples.

It was found in both normal and cholecystonephrostomized animals that there was no concentration of vit. K by the liver. This is in direct contradiction to the previous widely accepted belief that vit. K goes to the liver. Andrus and Lord(15) showed there was some antihemorrhagic factor present in liver. Since vit. K is not stored in the liver, an alternative hypothesis is that vit. K is material for prothrombin synthesis. If so, one would expect to find some activity in the prothrombin moiety itself since the liver is the site of formation of most of the plasma proteins. However, this was not the case. It is also to be noted that there was no activity in the dog bone marrow, although it is believed that some prothrombin formation may take place there also(5). The only alternatives left are (i) that the labelled methyl group does not participate in the physiological action of vit. K or (ii) that as no storage occurs, vit. K activity is dependent directly on blood levels of the vitamin. The first alternative seems very unlikely in view of the fact that only compounds with the methyl group are active, although experiments with vit. K labelled in some other position would provide more direct evidence. A marked dependence of prothrombin level on vit. K absorption has been shown by Mann *et al.*(20). With drainage of all abdominal lymph, a marked fall in the blood prothrombin level resulted within 18 hours. If we assume that vit. K is the only factor affecting prothrombin removed in this latter experiment it indicates a very marked dependence indeed on blood levels.

Summary. C^{14}-menadione (2-methyl-C^{14}-1:4-naphthoquinone) was prepared and administered intramuscularly to mice in doses of 1 and 0.1 mg, and to cholecystonephrostomized dogs in a dose of 1 mg/kg. The radioactivity was rapidly absorbed from the injection site and excreted in the urine, with only traces being detected in the blood. Quantities of radioactive material were not stored in any tissue. With the largest dose (1 mg/25 g mouse) a trace of activity was found in the liver and lung. The activity in the urine was attached to a single compound but a second component appeared on mild hydrolysis of the urine.

We are greatly indebted to Dr. J. M. Pepper for supervising attempts of one of us (P.S.) to synthesize C^{14}-menadione, to Miss Erica Lepp, who performed the prothrombin times, to Miss R. Keeler, who supervised the operating technics, to Mr. D. Wilson for assisting with the radioactivity measurements, to Dr. G. J. Millar for the plasma protein fractionation, to Dr. James Campbell for instruction in catherization technics.

1. Dam, H., *Biochem. J.*, 1935, v29, 1273.
2. Schønheyder, F., *Biochem. J.*, 1936, v30, 890.
3. Greaves, J. D. and Schmidt, C. L., PROC. SOC. EXP. BIOL. AND MED., 1937, v37, 43.
4. Hawkins, W. B., and Brinkhous, K. M., *J. Exp. Med.*, 1936, v63, 795.
5. Baserga, A. and De Nicola, P., *Le Melattie Emorragiche. Societa Editrice Libraria,* Milan, 1950.
6. Brinkhous, K. M., *Medicine,* 1940, v19, 329.
7. Canessa, I. I., *Vitamina K. Imprenta La Sud-America,* Santiago, 1948.
8. Jaques, L. B. and Spinks, J. W. T., Trans. Third Conf. Josiah Macy, Jr. Foundation, *Blood Clotting and Allied Problems,* p. 68, 1950.
9. Phillips, R. V., Trevoy, L. W., Jaques, L. B., Spinks, J. W. T., *Can. J. Chem.*, in press, 1952.
10. Kapsinow, R., Engle, L. P., and Harvey, S. C.,

Surg. Gyn. and Obstet., 1924, v39, 62.

11. Markowitz, J., *Textbook of Experimental Surgery*, Wm. Wood, Baltimore, 1937.

12. Cohn, E. J., Gurd, F. R. V., Surgenor, D. M., Barnes, B. A., Brown, R. K., Deronaux, G., Gillespie, J. M., Hahut, F. W., Lever, W. F., Lin, C. H., Mittelman, D., Mouton, R. F., Schmid, K., and Uroma, E., *J. Am. Chem. Soc.*, 1950, v72, 465.

13. Lord, J. W., Andrus, W. D., Moore, R. A., *Arch. Surg.*, 1940, v41, 585.

14. Molitor, H., and Robinson, H. J., PROC. SOC. EXP. BIOL. AND MED., 1940, v43, 125.

15. Andrus, W. D., Lord, S. W., *J. Am. Med. Assn.*, 1940, v114, 1336.

16. Garnett, C., *J. Lab. Clin. Med.*, 1939, v24, 919.

17. Fieser, L. F., *J. Am. Chem. Soc.*, 1948, v70, 3151.

18. Richert, D. A., *J. Biol. Chem.*, 1944, v154, 1; 1951, v189, 763.

19. Doisy, E. A., Abs. Div. of Biol. Chem., *J. Am. Chem. Soc.* 116th meet. Sept. 66c, 1949.

20. Mann, J. D., Mann, F. D., Bollman, J. L., *Am. J. Physiol.*, 1949, v158, 311.

Received February 5, 1952. P.S.E.B.M., 1952, v79.

Effects of Intravenous Administration of Dextran on Renal Function.*† (19460)

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Expansion of the plasma volume produced by the intravenous infusion of human albumin and plasma is usually associated with an increased rate of renal plasma flow and occasionally with increased rates of glomerular filtration(1-3). Since it has been demonstrated that partially hydrolyzed dextran is an effective plasma volume expander(4-6), a study has been made of the effects of the intravenous administration of this substance on various renal functions.

Methods. Thirteen World War II male veterans, ages 27 to 34, who were normotensive, afrebrile, and well hydrated and who had no evidence of renal disease, were studied at rest and in the fasting state. Since the presence of large amounts of dextran interferes with the determination of inulin in plasma and urine, glomerular filtration rate was measured by the endogenous creatinine clearance, using Hare's creatinine method(7). Para-aminohippurate clearance (C_{PAH}) and measurements of tubular excretory mass (Tm_{PAH}) were performed as described by Goldring and Chasis(8), including bladder catheterization. Hematocrit determinations were done according to Wintrobe. In subjects 1-7 the procedure was as follows: After 2 control clearance periods, 500 cc of 6% dextran in normal saline‡ were given intravenously at the rate of 25 cc/min. Eight to 45 minutes after the termination of the infusion, 2 or more clearance periods were obtained. Subjects 8 and 9 received 1000 cc of 6% dextran; patient 10 was given 1500 cc; repeat clearances were begun immediately after the infusion was completed. In subjects 11-13, control clearances and Tm_{PAH} determinations were done on the first day. The following day 1000 cc of 6% dextran were administered intravenously, and the above procedures were then repeated 45 minutes to 2 hours following infusion. In 2 additional subjects a catheter was placed in the renal vein and simultaneous arterial and renal venous samples obtained for determination of PAH extraction before and immediately after the injection of 1000 cc of 6% dextran. All figures for clearances and Tm_{PAH} in the tables have been converted to a body surface area of 1.73 sq.m.

* Reviewed in the Veterans Administration and published with approval of the Chief Medical Director. Statements and conclusions of the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

† Aided by a grant from the American Heart Association.

‡ "Expandex," supplied by Commercial Solvents Corp., Terre Haute, Ind.