homogentisic acid or lead homogentisate had been added. Water solutions of these compounds likewise luminesce. Since there is no froth layer when water solutions are shaken, the addition of several drops of a froth-producing substance (*e. g.*, 2% egg albumin) greatly facilitates observation of the phenomenon as the froth layer, where the greatest oxidation no doubt occurs, gives off the most brilliant light.

It is also of interest that hydroquinone, which likewise oxidizes and blackens when treated in a similar manner, emits no light when treated similarly and thus differs in behavior from the substituted hydroquinone, homogentisic acid (hydroquinone acetic acid).

Summary. Alcaptonuric urines, as well as solutions of homogentisic acid or lead homogentisate, give off light when made alkaline and shaken with air.

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Effect of Congo Red on Plasma Prothrombin.*

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Congo red has been widely used as a hemostatic agent, but its mode of action remains obscure.[1] Wedekind, Becker and Wienert[2] first observed that Congo red injected intravenously had a hemostatic effect. The coagulation time was shortened. Blood platelets were increased 2 or 3 times the initial number by the 5th or 6th day and returned to the initial figure in 3 weeks. It has been suggested[1] that "the hemostatic action of Congo red may be related to a limitation of the function of the spleen, with resultant elevation of platelets in the blood." However, there are apparently no experimental studies that support such an opinion concerning the effect of Congo red.

Because of the reported beneficial results in controlling hemorrhage with Congo red, its effect on the level of plasma prothrombin was studied and compared to that of 2-methyl-1,4-naphthoquinone.

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1 Arons, I., and Sokoloff, B., *Am. J. Roent. and Rad. Ther.*, 1939, **41**, 834.

2 Wedekind, T., Becker, J., and Wienert, B., *Munchen Med. Wchnschr.*, 1930, **77**, 2049.

In order to obtain diminished plasma prothrombin levels mice were fed a diet containing mineral oil. It has recently been recorded that prothrombin deficiency in rats can be produced by feeding an adequate diet containing 20% by weight of mineral oil.[3]

In each experiment, the mice used were of the same stock and age (within one week). The diet of the control mice consisted of a mash of bread and milk, with dog biscuits in most experiments. Other mice received the same with mineral oil (C.P.) constituting 5, 10, or 20% by volume of the mash.

Mice were killed by ether and approximately 1.2 cc of blood (usually from 2 mice) was obtained by cardiopuncture and added to 0.2 cc of 1.5% sodium oxalate solution. The plasma prothrombin level was determined by the method of Warner, Brinkhous and Smith.[4] We are indebted to Miss Florence Stevens and Miss Virginia Hewitt of the Department of Surgery of the New York Hospital and Cornell University Medical College for these determinations.

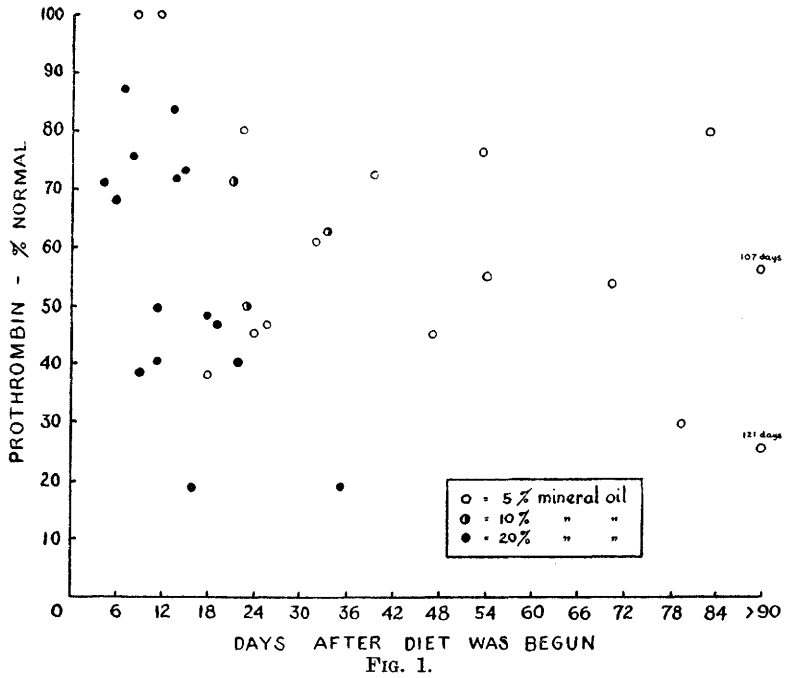


FIG. 1.

Plasma Prothrombin in Mice on a Diet Containing Mineral Oil.

[3] Elliot, M. C., Isaacs, B., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 240.

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

Effect of Mineral Oil in the Diet on the Plasma Prothrombin Level. Fig. 1 shows the plasma prothrombin level in mice at various intervals after receiving a diet containing mineral oil. As early as 4 days after mineral oil constituted 20% of the diet, a significant decrease in plasma prothrombin occurred. When 5% of the diet was mineral oil no change was noted until after the 12th day. The individual determinations varied considerably, but after the 3rd week plasma prothrombin levels were usually below 65% of normal. After receiving mineral oil in the diet for several weeks the mice, especially those receiving the large dose of mineral oil, lost varying amounts of hair, appeared poorly nourished, and were smaller than the controls.

Effect of 2-Methyl-1,4-Naphthoquinone on the Plasma Prothrombin Level. Mice with low plasma prothrombin levels were injected intraperitoneally on alternate days for from 2 to 4 times with 0.1 mg of 2-methyl-1,4-naphthoquinone in aqueous solution (0.1 cc). The mice were killed 1 to 2 days after the last injection. Table I shows that the prothrombin level in the blood was elevated to almost normal in most experiments, to normal in one, and to a higher level than the control in another experiment.

Effect of Congo Red on the Plasma Prothrombin Level. Mice with low plasma prothrombin levels, resulting from the addition of mineral oil to the diet, were injected intraperitoneally daily for from 3 to 8 days with 0.1 cc of 1% aqueous solution of Congo red.

TABLE I.

Effect of Intraperitoneal Injection of Congo Red and 2-Methyl-1,4-Naphthoquinone on Plasma Prothrombin Level of Mice on a Diet Containing Mineral Oil.

% mineral oil in diet	Days after feeding mineral oil mice killed	Prothrombin				
		Mineral oil alone	After Congo red		After 2-methyl-1,4-naphthoquinone	
		% normal	% normal	% raised	% normal	% raised
5	31	61	76	15	92	31
	39	73	73	0	100	27
	78	30	35	5	90	60
	83	80	85	5	—	—
	105	57	80	23	92	35
	119	26	57	31	—	—
20	12	50	90	40	—	—
	12	40	50	10	—	—
	14	84	96	12	—	—
	14	72	100	28	—	—
	15	73	84	11	100	27
	18	48	67	19	93	45
	19	47	89	42	100	53
	22	40	100	60	140	100
	22	40	90	50	—	—

The mice were killed on the day after the last injection. Table I shows that plasma prothrombin levels were as much as 60% (average 23%) greater among mice treated with Congo red than among mice on the diet containing mineral oil but which did not receive the chemical.

Two dogs whose prothrombin level had dropped conspicuously following cholecystonephrostomy received Congo red intravenously. In one dog the prothrombin level had dropped to 2% of normal 4 months after cholecystonephrostomy. After the intravenous administration of 5 cc of Congo red (1% aqueous solution) on 6 successive days the plasma prothrombin level rose to 15%. Twenty-four hours after the intravenous injection of aqueous 2-methyl-1,4-naphthoquinone the prothrombin level was 50%. In another dog the prothrombin level was 15% of normal 6 months after cholecystonephrostomy, and following the intravenous administration of 10 cc of Congo red on 2 successive days the plasma prothrombin was 25% of normal.

Discussion. These studies show that in mice prothrombin deficiency can be produced by feeding an adequate diet containing mineral oil, as had been shown previously in rats.[3] With mice, a significant decrease in the level of plasma prothrombin was observed as early as 4 days after mineral oil constituted 20% of the diet. This supports the belief that, unlike most fat soluble vitamins, Vitamin K is not stored by the body in appreciable amounts.[5] A similar rapid decrease in plasma prothrombin has not been observed by others in mammals on a Vitamin K-deficient diet or in mammals with bile excluded from the gastrointestinal tract (*e. g.,* by cholecystonephrostomy or external biliary fistula). Since it has been shown that certain microörganisms, including the colon bacillus, are capable of synthesizing Vitamin K in food, feces or pure culture,[6] it may be that in these animals the bacterial flora of the intestinal tract synthesize sufficient Vitamin K to prevent the rapid decrease in plasma prothrombin. It is possible that in animals with external biliary fistulae or cholecystonephrostomies the addition to the diet of mineral oil or of chemicals that reduce the bacterial flora of the intestine (*e. g.,* sulfaguanidine) might result in a more rapid decline in plasma prothrombin levels. However, the opinion that the inclusion of mineral oil in the diet alone is sufficient to produce a significant change in the prothrombin level of all animals is not supported by a few experiments in which we kept 6 rabbits on a diet containing

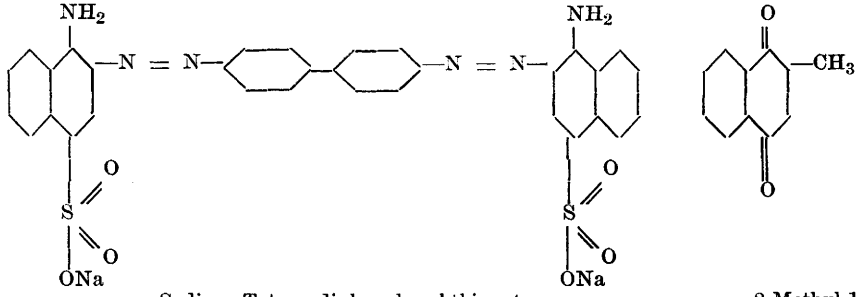
[5] Greaves, J. D., *J. A. M. A.*, 1939, **113**, 389.

[6] Almquist, H. J., Pentler, C. F., and Mecchi, E., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 336.

20% mineral oil (by volume) during a period of 4 weeks with no significant change in the prothrombin level.

The restoration of the prothrombin level to normal or almost normal following the administration of 2-methyl-1,4-naphthoquinone suggested that the diminished prothrombin levels of the mice on a diet containing mineral oil were not due to liver damage[7] but to failure of absorption of Vitamin K.

Many synthetic substances have Vitamin K activity and it is believed that the 1,4-naphthoquinone structure is most essential.[8] However, derivatives of active 1,4-naphthoquinone such as hydroquinones, quinhydrones, hydroquinone esters, or even 1,4-amino naphthols exhibit Vitamin K activity. The structural formula of Congo red is shown below with that of 2-methyl-1,4-naphthoquinone for comparison:



Sodium Tetrazodiphenylnaphthionate (Congo red)

2-Methyl-1,4-naphthoquinone

Most of the human cases in which Congo red presumably diminished or arrested bleeding were apparently not associated with a low prothrombin level,[1] and it is probable that the hemostatic action of Congo red is not confined to its Vitamin K-like effect. However, its beneficial effect on hemorrhage, especially in cases associated with jaundice in humans, may be due, at least in part, to its Vitamin K-like action in raising the plasma prothrombin above the level at which bleeding occurs.

Summary and Conclusions. The level of plasma prothrombin of mice can be lowered by the addition of mineral oil to the diet. The diminished level of plasma prothrombin can be raised significantly by Congo red and restored to normal by the parenteral administration of 2-methyl-1,4-naphthoquinone. The hemostatic action of Congo red may be explained in some cases, at least in part, by its Vitamin K-like action in raising the plasma prothrombin level.

[7] Lord, J. W., and Andrus, W. DeW., *Arch. Surg.*, 1941, **42**, 643; *Arch. Int. Med.*, 1941, **68**, 199.

[8] Freeman, S., and Grodius, F. S., *Surg. Gyn. and Obs.*, 1941, **72**, 417.