ponents were not detected. In place of Nos. 4 and 7, there were 2 new peaks in the feces with slightly increased retention times. This suggests that the 2 active CEF components are metabolized in the liver and excreted into the intestine *via* the bile, both in the chick and the rat. No CEF-like material was found in kidneys, adrenals, or urine.

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Oxygen Consumption and NADPH Oxidation in Microsomes from Vitamin K-Deficient, Warfarin- and Dicumarol-Treated Rats.* (31030)

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In the pathway of electron transport for cytochrome P450 reduction and activation of oxygen in mixed function oxidases, a related reaction, that of lipid peroxidation, has been described. When reduced nicotinamide adenine dinucleotide phosphate (NADPH) added to rat liver microsomes was oxidized, the consumption of oxygen was greatly enhanced by various pyrophosphates in the presence of ferric iron(1,2,3,4,5). Oxygen consumption with concurrent lipid peroxidation by microsomes has been observed upon the addition of only ferrous iron, suggesting that lipid peroxidation is catalyzed by a pyrophosphate chelate of $Fe^{2+}O_2$(6). Lipid peroxidation is not inhibited by carbon monoxide(4).

In this paper, the effect of vitamin K deficiency upon oxygen consumption and lipid peroxidation in rat liver microsomes is reported. Since menadione is known to be an acceptor in the NADPH-cytochrome c reductase system(7,8), our investigations included the possible role of vitamin K in the lipid peroxidation pathway.

Materials and methods. Control (+K) rats were given vitamin K-deficient diets plus the diphosphosodium ester of menadione(9). The deficient group (−K) received the same diet without vitamin supplementation and were housed in tubular, coprophagy-preventing cages(10). Warfarin-treated (W) and dicumarol-treated (D) animals were controls injected with 20 mg of sodium warfarin or dicumarol per 100 g of body weight, 18-24 hours prior to sacrifice. All animals were fasted overnight.

The animals were killed by decapitation and plasma prothrombin times determined by the method of Quick(11). Livers§ were homogenized in 3 times their volumes of 0.25 M sucrose or 1.15% KCl,‖ and a fraction containing primarily heavy microsomes was obtained(12). Final suspensions were made in 1.15% KCl in 0.02 M Tris buffer, pH 7.4 at 37.5°C and contained 5 mg of microsomal protein per ml(13). Oxygen consumption at 37.5°C was measured by the Warburg technique and by an oxypolarograph with a vi-

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§ When livers were taken from treated animals and microsomal fractions prepared, livers were removed from control rats and handled similarly.

‖ Preliminary work on oxygen consumption was done in 0.25 M sucrose, but 1.15% KCl was substituted when malonaldehyde measurements were made(1).

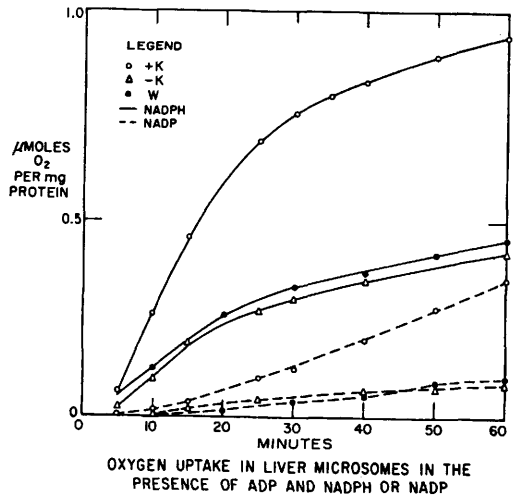

FIG. 1. Oxygen consumption in the Warburg. 3 ml-system containing 5 mg of microsomal protein; 1 μmole of NADPH or NADP; and 5 μmoles of ADP in KCl-Tris buffer.

brating platinum electrode. Malonaldehyde was determined according to Kohn and Liversedge(14) and demethylation of codeine according to Axelrod(15). NADPH oxidation was followed at 340 mμ and the reduction of cytochrome c at 550 mμ in the Perkin-Elmer Model 202 Spectrophotometer(16).

Results. Fig. 1 summarizes the Warburg results on oxygen consumption by microsomes. With NADPH, oxygen consumption in microsomes from control (+K) rats was markedly higher than in microsomes from deficient (−K) and warfarin-treated (W) animals. Microsomes from dicumarol-treated (D) animals followed the same pattern as those from W animals. Prothrombin times exceeded 40 seconds for vitamin K-deficient and drug-treated rats, but the degree of depression of oxygen consumption correlated poorly with the lengthening of prothrombin time.

When NADP replaced NADPH in the system, oxygen consumption by W and −K microsomes was very low (Fig. 1). For +K microsomes, oxygen consumption appeared to increase with time, although no measurable NADPH production by microsomes has been found in this system. Oxygen consumption by +K microsomes which were incubated with NADPH, ADP, and warfarin (1×10^{-5} M to 1×10^{-3} M) or dicumarol (1×10^{-5} M) was not depressed.

Since a −K or drug-treated animal may not eat as well as a +K rat, we studied oxygen consumption by microsomes from +K, −K, W, and D animals when half of the animals from each group were allowed only water for 48 hours and the other half 10% glucose in water.¶ Although oxygen consumption by microsomes from rats given only water was slightly less than from those given 10% glucose, it was proportionally higher in microsomes from +K than from −K, W or D animals.

In microsomal samples incubated 10 minutes, the color developed with the thiobarbituric acid reagent was usually depressed in the deficient (20-78%), dicumarol-treated (11-48%) and warfarin-treated (0-50%) animals with respect to their controls. In contrast, microsomal demethylation of codeine was not decreased.

In Fig. 2, the results of experiments on NADPH oxidation and oxygen consumption on the same microsomes are given for +K and −K animals. The addition of menadione to +K or −K microsomes containing NADPH stimulated NADPH oxidation more rapidly than did ADP and there were no differences in the oxidation rates between +K and −K microsomes when the menadione was present. Similar results were observed when

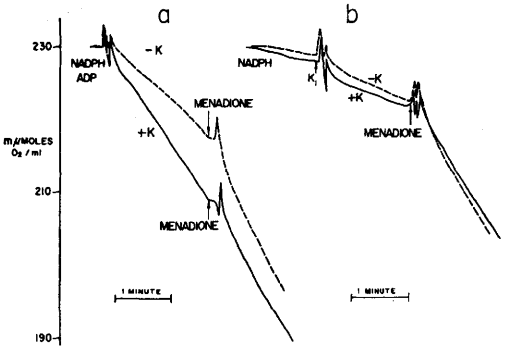

FIG. 2. Oxygen consumption in the Warburg and NADPH oxidation. Systems in KCl-Tris buffer; μmoles per mg of microsomal protein; NADPH, 0.2; ADP, 1; vit K_3, 0.01.

¶ Twenty, 10 and 5 mg of dicumarol or warfarin per 100 g of body weight were given to controls 60, 40 and 20 hours, respectively, before sacrifice.

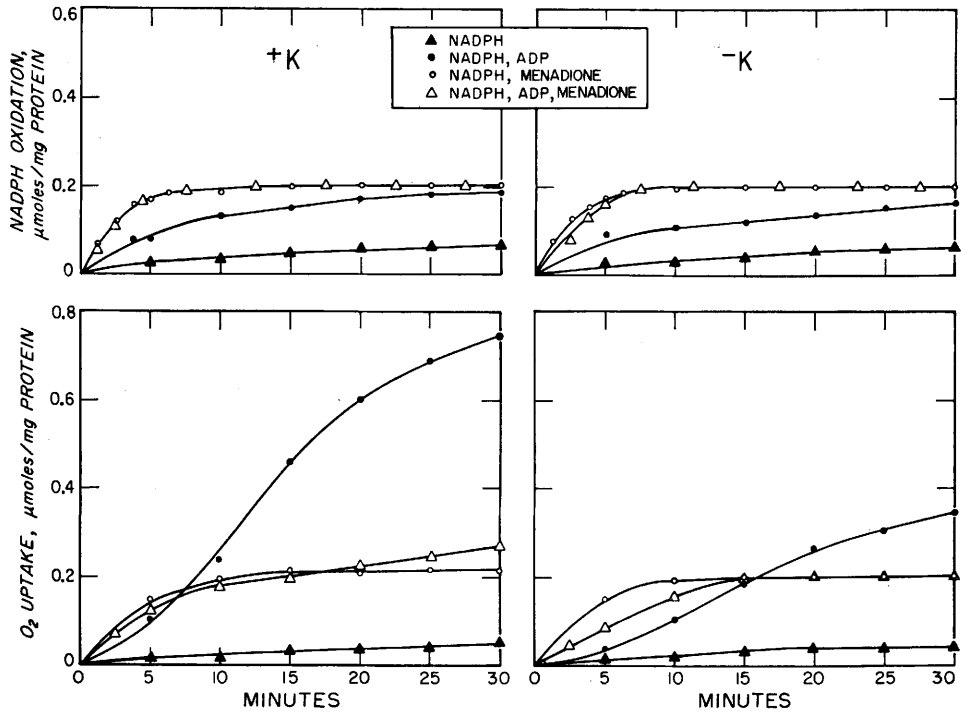

FIG. 3. Oxygen consumption in the polarograph. System, 2.5 ml; NADPH, 0.8 μmole; ADP, 2.5 μmoles; microsome, 1.0 mg protein; vit K_3, 0.02 μmole; vit K_1, 0.02 μmole.

cytochrome c replaced menadione. The stimulation with menadione was insensitive to dicumarol (10^{-4}), warfarin (10^{-4} M), KCN (10^{-4} M), nitrofurantoin (10^{-4} M), and chloropromazine (10^{-4} M), but was sensitive to p-chloromercuribenzoate (1.6×10^{-4} M) in both +K and −K microsomes (80% inhibition).

During a 30-minute incubation of a NADPH-microsomal mixture (Warburg technique), the stimulation of oxygen consumption by ADP, over that theoretically expected from oxidation of NADPH, did not occur when menadione was added (Fig. 2). In controls, microsomal lipid peroxidation and demethylation of codeine were inhibited 99 and 75%, respectively, by menadione (10^{-5} M).

As shown by the oxypolarograph data (Fig. 3a), the rate of oxygen consumption by +K microsomes was immediately more rapid than by −K microsomes upon addition of NADPH and ADP. Subsequent addition of menadione did not increase noticeably the rate of oxygen uptake by +K microsomes, but that by −K microsomes quickly paralleled the rate for the controls. Vitamin K_1 in equal molar quantities could not replace menadione in markedly stimulating oxygen consumption in −K microsomes after the addition of NADPH and ADP. However, vit. K_1 affected oxygen uptake in the same manner as did menadione (Fig. 3b) but to a much lesser extent.

In all the experiments, the stimulation of oxygen consumption by ADP in NADPH-microsomal systems was increased negligibly by addition of ferrous iron presumably because iron, as a contaminant of the commercial ADP, was present in adequate amounts (3).

Discussion. Microsomal lipid peroxidation was decreased in vit. K deficiency whereas the demethylation of codeine was not. That this diminution and the corresponding decrease in oxygen consumption in this reaction does not merely reflect general lipid deprivation in a vit. K-deficient rat is supported as follows: (1) Microsomes from warfarin- and dicumarol-treated animals showed similar decreases; (2) the experiments after 48-hour periods of food withdrawal gave the

same results. In addition it is well known that vit. K-deficient rats are not "starved" animals.**

The transport of electrons, NADPH-cytochrome c reductase to menadione or to other artificial acceptors as 2,6 dichlorophenolindophenol(5,17) and cytochrome c was not inhibited in microsomes from vit. K-deficient rats. Vitamin K_1 when added to the microsomal systems appeared to act as menadione, *i.e.*, as an artificial acceptor, although to a much lesser extent. This may be attributed to not only the relative ease with which menadione can attain an active site compared to vit. K_1, but also to the rapidity with which the semiquinone of menadione can be reoxidized by molecular oxygen.

The effect of vit. K deficiency appears only in the autocatalytic process itself. Thus, the immediate possibilities for investigation are changes in the composition of lipids or the production or increase of an inhibitor to lipid peroxidation.

Summary. Oxygen consumption relative to lipid peroxidation was decreased in microsomes from vitamin K-deficient, warfarin-treated, and dicumarol-treated rats. Vitamin K_1, when added to the microsomal system, appears to function similarly to the artificial acceptor menadione and does not alleviate the differences between control and vitamin K-deficient animals observed in lipid peroxidation.

** A rat on a vit. K-deficient diet will gain weight at a rate almost equal to that of a control. Twenty-four to 48 hours before death due to hemorrhage, the animal will stop eating.

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Gas-Liquid Radiochromatography of Intact Labeled Cholesterol Esters.* (31031)

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Gas-liquid radiochromatography is an excellent technique for simultaneous determination of mass and radioactivity of labeled components emerging from gas chromatographic columns(1-3). It offers several advantages over conventional techniques such as the direct determination of specific activity of one or more components and rapid analysis. It is particularly suited for metabolic studies where a number of components

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