Changes in Phylloquinone Epoxidase Activity Related to Prothrombin Synthesis and Microsomal Clotting Activity in the Rat

By ALLAN K. WILLINGHAM and JOHN T. MATSCHINER

Department of Biochemistry, University of Nebraska College of Medicine, Omaha, Neb. 68105, U.S.A.

(Received 21 January 1974)

The oxidation of phylloquinone to the 2,3-epoxide (by phylloquinone epoxidase) was studied in liver from control and warfarin-resistant rats. The reaction requires microsomal fraction, soluble protein, a heat-stable soluble factor and O_2 . It is not inhibited by CO or CN^- . Epoxidase activity was stimulated if plasma prothrombin was lowered either by anticoagulants or the absence of vitamin K. The activity of the enzyme rapidly returned to normal values after the administration of vitamin K to hypoprothrombinaemic rats. These differences in the activity of the enzyme occur in the microsomal fraction and not the cytosol. A thrombin-generating polypeptide that accumulates in microsomal fraction of hypothrombinaemic rats correlated directly with epoxidase activity. These data support the view that enzymic interconversion of phylloquinone and its 2,3-epoxide participates in the biological activity of vitamin K.

Studies with rats treated with warfarin have shown that phylloquinone 2,3-epoxide is a major metabolite of phylloquinone in the liver (Matschiner *et al.*, 1970). Phylloquinone is 2-methyl-3-phytyl-1,4-naphthoquinone or vitamin K_1 . The term, vitamin K, is used to refer to the various forms of the vitamin without regard to the structure of the side chain at carbon 3. The biological activity of phylloquinone epoxide appears to be dependent on its conversion into phylloquinone. Warfarin inhibits the reduction of epoxide to vitamin K *in vivo* (Bell & Matschiner, 1970; Bell *et al.*, 1972) and *in vitro* (Matschiner *et al.*, 1973). It has been proposed that warfarin inhibits the action of vitamin K by causing accumulation of the epoxide (Bell & Matschiner, 1972). These studies have led us to examine the nature of the epoxidation reaction *in vitro*.

Vitamin K may be involved in the conversion of a precursor protein into prothrombin in the rat (Bell & Matschiner, 1969; Ranhotra & Johnson, 1970; Shah & Suttie, 1971; Suttie, 1970, 1973). A material which produces thrombin activity after incubation with venom from *Echis carinatus* (Schieck *et al.*, 1972) accumulated in microsomal fraction of vitamin K-deficient rats and disappeared rapidly after the administration of vitamin K (Suttie, 1973). In the present report, the relationship of this precursor to phylloquinone epoxidase activity was also studied.

Experimental

Materials

[^{14}C]Phylloquinone (70 mCi/mmol), uniformly labelled in the phytyl side chain, was prepared as described by Matschiner (1970). The vitamin was

purified by chromatography on silicic acid and dissolved in ethanol. Sodium warfarin was the gift of Endo Laboratories, Garden City, N.Y., U.S.A. *Echis carinatus* venom and nicotinamide nucleotides were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. Haematin, tetrahydropteridine and sodium ascorbate were obtained from Calbiochem, La Jolla, Calif., U.S.A. Plastic plates precoated with silica gel G and fluorescent indicator were obtained from Brinkmann Instruments, Westbury, N.Y., U.S.A. [^{14}C]Taurodeoxycholate was obtained from Mallinckrodt Chemical Co., St. Louis, Mo., U.S.A.

Animals

Male and female rats (8–10 weeks old) of a Sprague-Dawley-derived strain were obtained from a local supplier. Homozygous warfarin-resistant rats were obtained by sibling mating from breeders given by Dr. Judith Pool (Pool *et al.*, 1968). Rats were fed on Purina Laboratory Chow (Purina Mills, St. Louis, Mo., U.S.A.), except for those experiments in which vitamin K was restricted. Purified diet, deficient in vitamin K, was purchased from General Biochemicals, Chagrin Falls, Ohio, U.S.A. The deficient diet contained 21% (w/w) soya protein, 43% (w/w) corn starch, 22% (w/w) glucose monohydrate, 5% (w/w) corn oil and an adequate supply of minerals and vitamins except for vitamin K (Matschiner & Doisy, 1965).

Clotting assay

The concentration of plasma prothrombin was determined by the method of Hjort *et al.* (1955). Blood was obtained by cardiac puncture. Control

plasma was obtained from 10 or more normal control male rats 13–14 weeks of age.

Epoxidase preparation

Rats were decapitated and the livers quickly excised and chilled. The livers were minced and homogenized by using a Polytron 20ST (Brinkmann) homogenizer at low speed. Homogenates (25%, w/v) were prepared in 0.25 M-sucrose–0.05 M-potassium phosphate buffer, pH 7.5, unless otherwise stated. Homogenates were centrifuged at 10000–15000g for 20 min in a Sorvall RC2-B centrifuge for preparation of supernatant fractions. Microsomal fractions and cytosol were prepared from 15000g supernatant by centrifugation at 105000g for 1 h in a Beckman L3-50 ultracentrifuge. The microsomal pellet was washed once and suspended in fresh homogenizing medium at one-half the volume of the 15000g supernatant. Soluble protein was prepared from cytosol by desalting techniques with Sephadex G-25. Cytosol (20 ml) was passed through a column of Sephadex G-25 (2.5 cm diam., 100 ml bed volume). The protein that moved with the void volume was collected and used in these studies. The fraction used contained 98% of the added protein and 1.5 ml was equivalent to 1.0 ml of cytosol. Protein concentration was determined by the method of Lowry *et al.* (1951) with a Lab-Trol standard (Dade Division, American Hospital Supply, Miami, Fla., U.S.A.).

Assay for epoxidase

All incubation mixtures contained 5–10 μ M-sodium warfarin to inhibit vitamin K epoxide reductase. [14 C]Phylloquinone was added in 50 μ l of ethanol to initiate the epoxidation reaction. Incubations were carried out in open flasks at 37°C in a Dubnoff incubator with constant shaking. Reactions were stopped by the addition of 7 ml of propan-2-ol-hexane (3:2, v/v). The mixture was transferred to stoppered tubes, shaken vigorously and centrifuged at 1500 rev./min for 10 min. The hexane extract contained 80–100% of the added radioactivity. [14 C]Phylloquinone and phylloquinone epoxide were separated from the extract with carrier on partition t.l.c. described by Matschiner *et al.* (1970). The spots of epoxide and vitamin were detected under u.v. light. Plates were cut and transferred to scintillation vials for assay in a Packard model 3375 liquid-scintillation spectrometer. Radioactivity in the epoxide and vitamin by this procedure accounted for 90–100% of the chromatographed 14 C.

Precursor assay

The venom of *Echis carinatus* has been used to generate thrombin activity from prothrombin (Schieck *et al.*, 1972) and from microsomal extracts of livers

from hypoprothrombinaemic rats (Suttie, 1973). The following procedure was used in these studies to measure clotting activity from microsomal fractions. All steps except the final assay were carried out at 0–4°C. Microsomal fractions prepared as described above were suspended in fresh homogenizing medium at one-sixth the volume of the 15000g supernatant and treated with an equal volume of 2% sodium deoxycholate. The treated suspension was dialysed for 8 h against 0.025 M-Tris-HCl buffer, pH 9.0 and then for 10–12 h against 0.025 M-potassium phosphate buffer, pH 7.5. Material not solubilized was separated by centrifugation at 105000g for 1 h. A portion (0.2 ml) of the supernatant was incubated with 0.1 ml of *Echis carinatus* venom (1 mg/ml in water) at 37°C for 20 min. The timed clotting reaction was started by addition of 0.1 ml of BaSO₄-adsorbed ox plasma (same preparation used in assay for plasma prothrombin) and measured by using an automatic coagulation timer (Fibrometer, BBL, Division of Becton Dickinson and Co., Cockeysville, Md., U.S.A.). For each experiment, dilutions of the sample with the fastest clotting time per mg of protein were used to develop a standard curve to determine the activity of samples with slower clotting times. Data are presented as the percentage of maximum clotting activity and are corrected for the concentration of protein in the microsomal extract.

Results

Several cofactors for oxidative reactions, NADPH (1.0 mM), NADH (1.0 mM), NADP⁺ (1.0 mM), NAD⁺ (1.0 mM), Fe²⁺ (10 μ M), ascorbate (0.6 mM), tetrahydropteridine (0.6 mM), haematin (0.2 μ M), FMN (1 mM) and FAD (1 mM), did not stimulate the epoxidation of phylloquinone in homogenates of rat liver. The thiol reagent, dithiothreitol, had no effect at 3 mM and was inhibitory at 10 mM. CO, KCN and EDTA had little effect on the formation of epoxide. Maximum activity of the epoxidase required both the microsomal and soluble fractions of rat liver, i.e. the 12000g supernatant. In subsequent experiments microsomal fractions alone were inactive. The effect of CO on the reaction was studied under conditions permitting observed inhibition of a cytochrome P-450-dependent enzyme (Trülzsch *et al.*, 1973). The results of this study are shown in Table 1. The amount of phylloquinone epoxide formed in the presence and absence of CO was similar whereas the amount of taurocholic acid formed in the presence of CO was inhibited by 50–75%. To determine a requirement for O₂, incubations were carried out under N₂. The reaction was inhibited by 75% after bubbling N₂ through incubation mixtures for 1 min and quickly sealing the flasks after addition of vitamin K. The inhibition was reversible, since

Table 1. *Effect of CO on phylluquinone epoxidase and 7 α -hydroxylase of rat liver*

The livers from two rats were homogenized in 3 vol. of 0.25 M-sucrose containing 50 mM-potassium phosphate buffer, pH 7.5, 30 mM-nicotinamide and 1.5 mM-EDTA. Incubations contained 2 ml of 10000g supernatant, 0.5 μ mol of NADPH, 30 nmol of warfarin and either 40 nmol of [14 C]taurodeoxycholate or 1.56 nmol of [14 C]phylluquinone in a total volume of 3 ml.

Incubation time (min)	Product formed (% conversion)			
	-CO		+CO	
	Phylluquinone epoxide	Taurocholic acid	Phylluquinone epoxide	Taurocholic acid
5	18.1	11.7	18.4	3.4
10	25.7	18.4	23.3	4.5
20	31.8	25.5	30.3	14.0
30	37.4	38.4	33.9	13.7

Table 2. *Requirement for microsomal and soluble fractions in epoxide formation*

Liver fractions were prepared as described in the Experimental section. Incubations contained 1 ml of microsomal fraction (11.9 mg of protein), 2 ml of cytosol (37.6 mg of protein) or 3 ml of soluble protein (38.4 mg), 1.56 nmol of [14 C]phylluquinone and 30 nmol of sodium warfarin in a total volume of 6 ml. Boiled fractions were prepared by heating in a boiling-water bath for 5 min and centrifuging at 1500 rev./min for 10 min. The resulting supernatant was used. Soluble protein was prepared by passing cytosol through a column of Sephadex G-25 (see the Experimental section). Incubations were for 10 min at 37°C.

Incubation	Epoxide formed (% of control)
Microsomal fraction+cytosol	100
Boiled microsomal fraction+cytosol	5
Microsomal fraction+boiled cytosol	6
Microsomal fraction+soluble protein	20
Microsomal fraction+soluble protein +boiled cytosol	70
Microsomal fraction+cytosol+epoxide (20 nmol)	99

control activity was present in purged flasks left open to air during the incubation period.

The cellular components required for formation of phylluquinone epoxide are shown in Table 2. Heating either the microsomal fraction or cytosol in a boiling-water bath for 5 min resulted in loss of epoxidase activity. Soluble protein prepared by passing cytosol through a column of Sephadex G-25 (see the Experimental section) gave some epoxidase activity if added to microsomal fraction; but most of the activity of the complete system was obtained only by combining microsomal fraction, soluble protein and boiled cytosol. The epoxide at a concentration over 10 times that of the incubated vitamin did not inhibit the reaction.

Table 3. *Epoxidase activity versus protein concentration in 15000g supernatant from normal and vitamin K-deficient rats*

The 15000g supernatant was prepared from livers of two normal and two vitamin K-deficient rats (see the Experimental section). Incubations contained the supernatant, 1.56 nmol of [14 C]phylluquinone and 30 nmol of warfarin in a total volume of 3 ml. The concentration of protein in the supernatant was 22.6 mg/ml from normal and 22.3 mg/ml from vitamin K-deficient rat livers. The results are the means $\pm$ S.E.M. of triplicate incubation for 5 min.

Protein (mg)	Epoxide formed (nmol)	
	Normal	Vitamin K-deficient
5.6	0.07 $\pm$ 0.00	0.09 $\pm$ 0.00
11.2	0.14 $\pm$ 0.01	0.17 $\pm$ 0.02
22.4	0.23 $\pm$ 0.01	0.31 $\pm$ 0.03
33.6	0.30 $\pm$ 0.02	0.40 $\pm$ 0.01

The components of rat liver required for epoxidation of phylluquinone were similar to those reported for epoxidation of squalene (Yamamoto & Bloch, 1970a,b). NADPH did not stimulate the initial rate of epoxidation of phylluquinone as was reported for squalene, but the concentration of squalene used was 70 μ M whereas the concentration of phylluquinone in the experiments reported here was never more than 0.5 μ M. Under these conditions the effect of exogenous NADPH may not be observed.

The activity of phylluquinone epoxidase was examined in liver from vitamin K-deficient as well as normal rats (Table 3). Epoxidase activity was higher in deficient rats especially if the enzyme was assayed at higher protein concentrations. These studies were extended to female rats of the control strain and male and female warfarin-resistant rats, which have a higher requirement for vitamin K (Hermodson *et al.*, 1969). The results are shown in Fig. 1. Vitamin K-deficient control males and warfarin-resistant males had the highest epoxidase activity. Plasma prothrombin concentration in these animals was less

Fig. 1. Phyloquinone epoxidase activity in control (a) and warfarin-resistant (b) rats with different plasma prothrombin concentrations

●, Females; ■, males; □, control males fed on vitamin K-deficient diet. Plasma prothrombin concentrations in these animals were determined by using pooled plasma from 13-week-old male control rats for reference values. The following concentrations were observed: control females, 107% of the reference value; control males, 90%; control males fed on vitamin K-deficient diet, <20%; warfarin-resistant females, 70%; warfarin-resistant males, <20%. Incubations contained 2 ml of 15000g supernatant from homogenates of liver, 30 nmol of sodium warfarin and 1.56 nmol of [14 C]phyloquinone in a total volume of 3 ml. The results are the average of two to nine experiments. Each incubation contained 46 ± 2 mg of protein. All rats were fed on Purina Laboratory Chow, except vitamin K-deficient control males, which were fed on purified diet.

Table 4. Role of microsomal fraction in epoxide formation in normal and vitamin K-deficient rats

Liver fractions were prepared from two normal and two vitamin K-deficient male rats (see the Experimental section). Incubations contained 1 ml of microsomal fraction, 2 ml of cytosol, 1.56 nmol of [14 C]phyloquinone and 30 nmol of warfarin in a total volume of 3 ml. Incubations were for 5 min. The results are the means $\pm$ S.E.M. of four incubations from two separate experiments. Protein concentration of the microsomal suspension was 11.1 mg/ml from normal and 10.7 mg/ml from vitamin K-deficient rat livers. The values for cytosol were 20.4 and 21.4 mg/ml respectively.

Microsomal fraction	Cytosol	Epoxide formed (nmol)
Normal	Normal	0.21 ± 0.01
Normal	Deficient	0.21 ± 0.02
Deficient	Normal	0.39 ± 0.03
Deficient	Deficient	0.32 ± 0.03

than 20% of normal. Normal control male rats fed on Purina Chow had higher epoxidase activity than control females fed on the same diet. Male rats have lower plasma prothrombin concentration than females (Matschiner *et al.*, 1967). Epoxidase activity of warfarin-resistant females fed on Purina Chow was also lower than that of resistant males fed on the

same diet. In all cases epoxidase activity was higher in vitamin K-deficient rats and inversely related to the concentration of plasma prothrombin.

The difference in epoxidase activity between vitamin K-deficient and normal male rats was found to reside in the microsomal fraction (Table 4). Regardless of the source of cytosol, epoxidase activity was highest in those incubation mixtures containing microsomal fraction from deficient rats. The concentration of protein in the microsomal fraction from normal and deficient rats was essentially the same.

The data in Fig. 2 show that vitamin K epoxidase activity increased after the administration of warfarin (25 μ g/100g) with a corresponding decrease in plasma prothrombin. Epoxidase activity was approximately doubled 4h after administration of warfarin and returned to control values after 60h. Plasma prothrombin concentration fell to 45% of normal 8h after warfarin treatment and slowly returned to normal values. This experiment was repeated with warfarin-resistant rats which were given an ineffectual dose of warfarin. No change was observed in plasma prothrombin or epoxidase activity.

The effect of phyloquinone on elevated epoxidase activity in vitamin K-deficient rats is shown in Fig. 3. Control male rats were fed on vitamin K-deficient diet until plasma prothrombin concentrations fell to 30% of normal. Warfarin-resistant male rats were fed

on Purina Laboratory Chow, which results in plasma prothrombin concentrations less than 20% of normal. At 15 min after injection of 5 μ g of phyloquinone to control rats or 100 μ g of phyloquinone to warfarin-resistant rats, epoxidase activity was decreased by 70% and 50% respectively. No prothrombin appeared in plasma during the first 15 min, but increased thereafter in both control and warfarin-resistant rats. Epoxidase activity after 30 and 60 min was approximately the same as that found in rats with normal concentrations of plasma prothrombin.

Precursor activity was measured in liver microsomal fraction of rats with various epoxidase activities and plasma prothrombin concentrations. Results with control male rats are shown in Fig. 4. After 2 days on vitamin K-deficient diet the epoxidase activity more than doubled and was high during the next 5 days, whereas plasma prothrombin decreased to 20% of normal. Precursor activity was undetectable at the start of the experiment and reached a maximum value after 2 days on vitamin K-deficient diet. It remained

Fig. 2. Changes in epoxidase activity and plasma prothrombin after administration of warfarin

Male rats fed on vitamin K-deficient diet supplemented with 2 μ g of phyloquinone/g were injected intraperitoneally with 25 μ g of sodium warfarin/100 g body wt. Plasma samples were obtained just before the rats were killed. At each time-point livers from two rats were combined and homogenized. Incubations contained 2 ml of 15000g supernatant, 30 nmol of warfarin and 1.56 nmol of [14 C]-phyloquinone in a total volume of 3 ml. Prothrombin values are the average of duplicate samples. ●, Epoxidase; ▲, prothrombin.

Fig. 3. Changes in epoxidase activity and plasma prothrombin after administration of phyloquinone to deficient control (a) and warfarin-resistant (b) male rats

Control male rats fed on vitamin K-deficient diet were injected intravenously with 5 μ g of phyloquinone. Warfarin-resistant male rats fed on Purina Laboratory Chow were injected intravenously with 100 μ g of phyloquinone. Plasma samples were obtained just before the rats were killed. At each time-point livers from two rats were combined and homogenized. Incubations contained 2 ml of 15000g supernatant, 30 nmol of sodium warfarin and 1.56 nmol of [14 C]phyloquinone in a total volume of 3 ml. Prothrombin values are the average of duplicate samples. ●, Epoxidase; ▲, prothrombin.

Fig. 4. Effect of removal of vitamin K and subsequent administration of phyloquinone on epoxidase activity, precursor and plasma prothrombin concentration

Male rats were fed on vitamin K-deficient diet for 7 days. After 7 days the animals were injected intravenously with $5\mu\text{g}$ of phyloquinone. Plasma samples were obtained just before the rats were killed. At each time-point livers from two rats were combined and homogenized. Precursor activity was measured in liver microsomal fractions treated with deoxycholate by using *Echis carinatus* venom (see the Experimental section). Incubations contained 2 ml of 15000g supernatant, 30 nmol of warfarin and 1.56 nmol of [^{14}C]phyloquinone in a total volume of 3 ml. Prothrombin values are the average of duplicate samples. ●, Epoxidase; ▲, prothrombin; ○, precursor clotting time (s).

high after 4 and 7 days. After 7 days, rats were injected intravenously with $5\mu\text{g}$ of phyloquinone and, as before, epoxidase activity and plasma prothrombin changed inversely. Precursor activity fell concomitantly with the activity of the epoxidase and was undetectable 30 min after administration of phyloquinone. This experiment was repeated with male and female warfarin-resistant rats with similar results.

Discussion

Earlier studies on the enzymic reduction of phyloquinone epoxide showed that the reductase is inhibited by warfarin in control rats and inhibited less in warfarin-resistant rats (Matschiner *et al.*, 1973). The reduction of vitamin K epoxide in rat liver microsomal fractions is considerably more active than the epoxidase reaction and does not vary under the conditions studied in this report (A. K. Willingham & J. T. Matschiner, unpublished work). Together with

the data presented here these results support the view that the enzymic interconversion of phyloquinone and its 2,3-epoxide is involved in the vitamin K-dependent production of prothrombin. If the thrombin-generating material of liver microsomal fraction is the precursor of prothrombin, epoxidation of vitamin K may be coupled with conversion of the precursor into prothrombin as shown in Scheme 1. A similar scheme was proposed by Ernster *et al.* (1972). The reductase appears to have a regenerative function allowing prothrombin synthesis to occur at low concentrations of vitamin K.

Abnormalities in the cyclic interconversion of vitamin K and the epoxide could explain impaired synthesis of the vitamin K-dependent clotting proteins under a variety of circumstances. A new proposal for the mechanism of action of warfarin based on inhibition of the reductase has been made (Bell & Matschiner, 1972; Matschiner *et al.*, 1973). Recent studies suggest that other anticoagulants, the chloro

Scheme 1. Involvement of vitamin K epoxidase in the formation of prothrombin

analogue of vitamin K (Lowenthal, 1970) and tetra-chloropyridinol (Marshall, 1972) may inhibit the synthesis of vitamin K-dependent clotting proteins by inhibiting the epoxidase (Willingham *et al.*, 1974).

We thank Mrs. M. Goldsmith, Mr. D. Schmidt and Mr. M. Rowley for their skilled technical assistance. This study was supported by Grant AM 14937 and Contract NICHD 72-2787 from the National Institutes of Health and the Nebraska Heart Association.

References

- Bell, R. G. & Matschiner, J. T. (1969) *Arch. Biochem. Biophys.* **135**, 152-159
- Bell, R. G. & Matschiner, J. T. (1970) *Arch. Biochem. Biophys.* **141**, 473-476
- Bell, R. G. & Matschiner, J. T. (1972) *Nature (London)* **237**, 32-33
- Bell, R. G., Sadowski, J. A. & Matschiner, J. T. (1972) *Biochemistry* **11**, 1959-1961
- Ernster, L., Lind, C. & Rase, B. (1972) *Eur. J. Biochem.* **25**, 198-206
- Hermanson, M. A., Suttie, J. W. & Link, K. P. (1969) *Amer. J. Physiol.* **217**, 1316-1319
- Hjort, P., Rapaport, S. I. & Owren, P. (1955) *J. Lab. Clin. Med.* **46**, 89-97
- Lowenthal, J. (1970) in *The Fat Soluble Vitamins* (Suttie, J. W. & DeLuca, H. F., eds.), pp. 431-446, University of Wisconsin Press, Madison
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Marshall, F. N. (1972) *Proc. Soc. Exp. Biol. Med.* **139**, 223-227
- Matschiner, J. T. (1970) in *The Fat Soluble Vitamins* (Suttie, J. W. & DeLuca, H. F., eds.), pp. 377-397, University of Wisconsin Press, Madison
- Matschiner, J. T. & Doisy, E. A., Jr. (1965) *J. Nutr.* **86**, 93-99
- Matschiner, J. T., Hsia, S. L. & Doisy, E. A., Jr. (1967) *J. Nutr.* **91**, 299-302
- Matschiner, J. T., Bell, R. G., Amelotti, J. M. & Knauer, T. E. (1970) *Biochim. Biophys. Acta* **201**, 309-315
- Matschiner, J. T., Zimmerman, A. & Bell, R. G. (1973) *Thromb. Diath. Haemorrh.* in the press
- Pool, J. G., O'Reilly, R. A., Schneiderman, L. J. & Alexander, M. (1968) *Amer. J. Physiol.* **215**, 627-631
- Ranhotra, G. S. & Johnson, B. C. (1970) *Life Sci.* **9**, 79-86
- Schieck, A., Kornalik, F. & Habermann, E. (1972) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **272**, 402-416
- Shah, D. V. & Suttie, J. W. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 1653-1657
- Suttie, J. W. (1970) *Arch. Biochem. Biophys.* **141**, 571-578
- Suttie, J. W. (1973) *Science* **179**, 192-194
- Trülsch, D., Greim, H., Czygan, P., Hutterer, F., Schaffner, F., Popper, H., Cooper, D. Y. & Rosenthal, O. (1973) *Biochemistry* **12**, 76-79
- Willingham, A. K., Laliberte, R. L., Bell, R. G. & Matschiner, J. T. (1974) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **33**, 672
- Yamamoto, S. & Bloch, K. (1970a) *Biochem. Soc. Symp.* **29**, 35-43
- Yamamoto, S. & Bloch, K. (1970b) *J. Biol. Chem.* **245**, 1670-1674