TABLE II.
Results of Adding Dried Whole Milk Following Periods of Butter Yellow-Rice Diet, on the Production of Liver Cancer.

No. of animals used	No. of days fed on butter yellow and rice	No. of days fed rice and Klim diet subsequent to butter yellow-rice diet	Liver changes*				
			—	±	+	++	+++
20	62	113-168	18	0	0	0	2
27	88	100-250	1	0	0	5	21
14	124	82-254	0	0	2	6	6

* See Table I for explanation of symbols.

induced in rats by feeding unpolished rice and *p*-dimethylaminoazobenzene. 2. The production of liver cancer in rats fed *p*-dimethylaminoazobenzene was partially inhibited by the daily ingestion of dried whole milk (50% protection at 200 days). 3. Liver cirrhosis produced by *p*-dimethylaminoazobenzene has been treated successfully by a rice diet containing 15% dried whole milk. (Based on previous evidence of the high incidence of liver cirrhosis in rats produced by *p*-dimethylaminoazobenzene-rice diet.[11]). 4. Once adenomatous hyperplasia of bile ducts, cholangioma, or hepatoma had been established in the livers, these benign and malignant tumors could not be destroyed by ingestion of the rice-milk diet.

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14765

Effect of Malonate, Fumarate, Succinate, and Citrate on Synthesis of Acetylcholine *in vitro*.*

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The increased synthesis of acetylcholine *in vitro* under aerobic conditions is greater in the presence of serum and spinal fluid obtained from healthy humans than from patients with myasthenia gravis.[1,2,3] Since the synthesis of acetylcholine *in vitro* is increased in the presence of glucose and pyruvate[4] the following experiments were designed to ascertain whether or not possible intermediates of glucose and pyruvate metabolism modify the synthesis of acetylcholine.

Method. The synthesis of acetylcholine was studied by the method of Quastel, Tennenbaum, and Wheatley[4] with minor modifications.[2] Varying amounts of sodium succinate, sodium fumarate, sodium malonate, sodium citrate, and sodium oxalate were added to mixtures containing 100 mg minced fresh frog brain, 1 cc serum or spinal fluid, 3 mg physostigmine salicylate, and 2 cc Ringer's solution. The pH of the mixtures was adjusted to 7.4. Identical mixtures without the salts of these organic acids served as controls. The mixtures were shaken and incubated aerobically for 4 hours at 37°C. After incubation the amounts of free and total acetylcholine synthe-

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1 Torda, C., and Wolff, H. G., *Science*, 1943, **98**, 224.

2 Torda, C., and Wolff, H. G., *J. Clin. Invest.*, 1944, **23**, 649.

3 Torda, C., and Wolff, H. G., *Science*, 1944, **100**, 200.

4 Quastel, J. H., Tennenbaum, M., and Wheatley, A. H. M., *Bioch. J.*, 1936, **30**, 1668.

TABLE I.
Effect of the Substances on the Synthesis of Acetylcholine *in vitro*.

Substance	Final concentrations in mols	Amount of acetylcholine synthesized in % of control*			
		Free acetylcholine In the presence of frog brain and		Total acetylcholine In the presence of frog brain and	
		Spinal fluid	Serum	Spinal fluid	Serum
Sodium malonate	8×10^{-5}	66	70	64	62
	8×10^{-4}	55	54	54	61
	8×10^{-3}	53	50	51	58
" fumarate	8×10^{-5}	131	134	125	126
	8×10^{-4}	138	140	130	130
	8×10^{-3}	142	151	140	147
" succinate	5×10^{-5}	143	136	140	136
	5×10^{-4}	151	150	155	149
	5×10^{-3}	145	142	148	143
" citrate	3.5×10^{-5}	130	135	129	128
	3.5×10^{-4}	140	142	139	138
	3.5×10^{-3}	150	154	150	147
" oxalate	4×10^{-5}	92	88	102	99
	4×10^{-4}	105	110	95	98
	4×10^{-3}	100	103	107	109

* Each value represents the average of 10 separate experiments. The S.E. of the mean for each value was less than ±8%. The amounts of acetylcholine synthesized in μg per 100 mg frog brain, followed by the S.E. of the mean were: spinal fluid: free, 1.57 ± 0.048; total, 2.36 ± 0.052; serum: free, 1.15 ± 0.045; total, 1.69 ± 0.054.

sized were assayed biologically on the sensitized rectus abdominis muscle of the frog. Since the compounds in the concentrations used did not modify the effect of acetylcholine in inducing muscle contraction, this procedure should be a valid indicator of the acetylcholine content of the mixtures.

Results. The amounts of acetylcholine synthesized in the presence of the substances used are given in Table I. Sodium malonate decreased the amount of acetylcholine synthesized, while succinate, fumarate, and citrate increased the synthesis of acetylcholine.

To determine whether or not the increased synthesis with sodium citrate was due to the removal of calcium ion, sodium oxalate was added in concentrations large enough to remove more calcium than did citrate, but still small enough to be bound mainly by the calcium present in the mixtures and therefore without any effect of its own upon the synthesis of acetylcholine. Since the synthesis of acetylcholine was not significantly modified in the presence of sodium oxalate in the concentrations used, the effect of sodium citrate was not due to decreased calcium ion concentration in the incubated mixtures.

Discussion. In the presence of the above intermediates of glucose and pyruvate metabolism in small concentrations the synthesis of acetylcholine was increased by about 50%. Succinate in larger concentrations and under different oxygen tension may have a different effect.[4,5,6] Malonate, known to depress the oxidation of succinate,[7,8] also depresses the synthesis of acetylcholine.

For the mechanism of action of fumarate, succinate, and citrate it is possible that these substances in small concentrations increase the synthesis of acetylcholine because they are

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converted to some compounds[9] that can be utilized for the acetylation of choline or because of energy liberated during the metabolism of fumarate, citrate, and succinate.

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Summary. 1. The synthesis of acetylcholine *in vitro* in the presence of sodium malonate, succinate, citrate, and fumarate was investigated. 2. Malonate decreased the synthesis of acetylcholine in the concentrations used, while citrate, succinate, and fumarate increased it.

14766

Effect of Vitamin K (Menadione) on Choline Esterase Activity, Acetylcholine Synthesis, and Striated Muscle.*

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Under physiological conditions menadione (2-methyl-1,4-naphthoquinone) has the ability to react with substances containing the –SH group.[1,2,3] Therefore the activity of some enzymes having an active –SH group may be modified by menadione. Since choline esterase[4] and the enzyme that synthesizes acetylcholine[5] probably contain an active –SH group, a study was made to ascertain whether or not menadione modifies the activity of these enzymes.[6]

Experimental. I. *Effect of menadione on the Synthesis of Acetylcholine.* The synthesis of acetylcholine was studied by the method of Quastel, Tennenbaum, and Wheatley[7] with minor modifications.[8] Varying amounts of menadione were added to mixtures containing 100 mg minced fresh frog brain, 3 cc Ringer's solution, 3 mg physostigmine salicylate and 4.8 mg glucose. The pH of the mixtures was adjusted to 7.4. Identical mixtures without menadione served as controls. The mixtures were shaken and incubated aerobically for 4 hours. After incubation the amounts of free and total acetylcholine synthesized were assayed biologically on the sensitized rectus abdominis muscle of the frog.

Menadione depressed the synthesis of acetylcholine and the decrease was more marked in the presence of larger concentrations of the vitamin (see Table I).

In another series of experiments the amount of substrate was increased by adding 1 cc of spinal fluid to the mixtures before incubation. In such mixtures more acetylcholine is synthesized.[9] In the presence of menadione the synthesis of acetylcholine was decreased; the percentage decrease being similar to that found in the absence of spinal fluid (Table I).

Methylene blue and menadione have similar effects on oxidative processes. Therefore the effect of methylene blue on the synthesis of acetylcholine was investigated. In the presence of 1×10^{-6} mol. methylene blue the synthesis of acetylcholine was 20% less (average of 6 series of experiments). Since methylene blue greatly increased the sensitivity of the

* This study was aided by a grant from the Josiah Macy, Jr., Foundation.

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7 Quastel, J. H., Tennenbaum, M., and Wheatley, A. H. M., *Bioch. J.*, 1936, **30**, 1668.

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