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.

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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

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

Concentration of menadione (mols)	Amounts of acetylcholine synthesized in μg per 100 mg frog brain			
	Free acetylcholine In the presence of frog brain and		Total acetylcholine In the presence of frog brain and	
	Ringer's solution*	Spinal fluid†	Ringer's solution*	Spinal fluid†
0	0.070 ± 0.023	1.57 ± 0.048	1.50 ± 0.044	2.36 ± 0.052
	Amounts of acetylcholine synthesized in percent of these controls.‡			
0	100	100	100	100
2×10^{-6}	86	83	83	84
2×10^{-5}	67	76	75	76
2×10^{-4}	61	61	70	63
2×10^{-3}	30	23	26	20

* Mixtures incubated at 23°C.
† Mixtures incubated at 37°C.
‡ Each value represents the average of 5 separate experiments. The S.E. of the mean for each value was less than ±5%.

eserinized rectus abdominis muscle to acetylcholine, the amounts of acetylcholine synthesized in the presence of larger concentrations of methylene blue are difficult to evaluate.

II. *Effect of Menadione on the Activity of Choline Esterase* (manometric method). Human serum or ox brains were used as a source of choline esterase. The brains were frozen and finely ground.[9] The tissue was pressed through muslin and suspended in bicarbonated Ringer's solution at pH 7.4. Two cc of the suspension of brain and menadione (from 0 to 0.774 mg) were placed in the body of the vessel of the Warburg apparatus and 0.2 cc of a 5% solution of acetylcholine in Ringer's solution was placed in the side cup. In another series of experiments instead of the suspension of brain 0.3 cc serum and 1.7 cc Ringer's solution were used. Mixtures without menadione and mixtures without menadione but containing physostigmine salicylate in various concentrations served as controls. The activity of the choline esterase was then ascertained manometrically by measuring the amount of CO_2 liberated during the hydrolysis of acetylcholine following the method of Ammon.[11]

Menadione did not modify the activity of choline esterase in concentrations from $1x10^{-7}$ to $1x10^{-3}$ mol., but in concentrations of $2x10^{-3}$ mol. the activity of choline esterase was depressed by 70% regardless of the origin of the enzyme (serum or brain). Under similar conditions a $5x10^{-7}$ mol. physostigmine salicylate solution caused a 70% inhibition (average of 20 series of experiments).

In the following it was ascertained whether or not menadione modifies the effect of acetylcholine and other chemical stimuli, such as potassium, in inducing muscle contraction.

III. *Menadione and the Effect of Acetylcholine in Inducing Muscle Contraction.* The rectus abdominis muscle of frog was excised and suspended in a muscle chamber containing 10 cc Ringer's solution. The muscle was then immersed in a solution of acetylcholine (50 μg per 100 cc Ringer's solution) for 2 minutes and the amount of contraction of the muscle was registered by an isotonic lever on a kymograph. The muscle was then washed with Ringer's solution for 10 minutes. This procedure was repeated until 3 successive exposures to the solution of acetylcholine gave similar responses. After this stabilization of the muscle was obtained, the muscle was washed for 5 minutes and immersed for 5 minutes in a series of Ringer's solutions containing menadione in increasing concentrations ($1x10^{-7}$ to $2x10^{-3}$ mol.).

The results are given in Table II. Menadione in concentrations from $1x10^{-7}$ to $1x10^{-3}$ mol. did not modify the effect of acetylcholine on the muscle. In concentrations of $2x10^{-3}$ mol. the effect of acetylcholine was increased (average 60%).

Methylene blue, a potent inhibitor of cho-

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TABLE II.
Effect of Menadione and Methylene Blue on the Contraction of Striated Muscle Induced by Acetylcholine and Potassium.

Concentration of the drugs in mols	Magnitude of contraction in percent of control.* Contraction induced with Acetylcholine		Potassium	
	In muscles immersed in Menadione	Methylene blue	In muscles immersed in Menadione	Methylene blue
0	100	100	100	100
1×10^{-7}	111	121	122	119
1×10^{-6}	104	213	182	157
1×10^{-5}	102	381	202	381
1×10^{-4}	108		295	
1×10^{-3}	99		464	
2×10^{-3}	162		470	

* Each value represents the average of 10 separate experiments. The S.E. of the mean for each value was less than ±5%.

line esterase,[12,13] increased the effect of acetylcholine on the rectus abdominis muscle in concentrations from 1×10^{-7} mol. (Table II).

IV. *Menadione and the Effect of Potassium in Inducing Muscle Contraction.* The rectus abdominis muscle of frog was prepared and contracted as described above except that a 20mM solution of potassium chloride was used instead of acetylcholine to induce a contraction of the muscle. The height of contraction induced by potassium cannot be compared to that induced by acetylcholine since these substances cause different physicochemical changes in the muscle cells.[14,15] The results given in Table II show that menadione significantly increased the magnitude of contraction induced by potassium. It is difficult to reverse the effect of menadione. Menadione and methylene blue similarly modify the effect of potassium (Table II).

V. *Effect of High Concentrations of Menadione on Striated Muscle.* 2.5×10^{-3} mol. solution of menadione initiated a muscle contraction in 3 minutes without an addition of any other agent. This concentration approximates a saturated solution of this substance. A similar contraction was induced by a 2.5×10^{-3} mol. methylene blue solution.

Discussion. The effects of menadione may be related to its ability to react with substances containing the –SH group;[1,2,3] its ability to inhibit the aerobic glycolysis;[3,16,17,18] and to its positive oxidation-reduction potential.[16] This assumption is based on a comparison of the effect of menadione with that of methylene blue.

The above results suggest that menadione may depress the activity of organs stimulated by acetylcholine since the synthesis of acetylcholine is depressed by low concentrations of this vitamin. This effect is probably not counteracted by the depression of the activity of choline esterase since the concentrations of menadione required for the latter are much higher. The sensitivity of the effector cells to some chemical stimuli may be increased by menadione, for example, the effect of potassium on striated muscle is increased several times. It is likely that menadione cannot occur in the striated muscle of the living body in concentrations high enough to induce contractions.

Summary. 1. The effect of menadione in concentrations from 1×10^{-7} to 2.5×10^{-3} mol. on the synthesis of acetylcholine, on the activity of choline esterase, and on the effect of some chemical agents in inducing muscle

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contraction was investigated. 2. In the presence of menadione the synthesis of acetylcholine is decreased. 3. The activity of choline esterase was not modified by concentrations of menadione up to $1x10^{-3}$ mol. and was decreased by higher concentrations (manometric method and striated muscle). 4. Menadione increased the effect of potassium in inducing muscle contraction. 5. In high concentrations ($2.5x10^{-3}$ mol.) it induced a contraction of the striated muscle. 6. The effect of methylene blue is similar to that of menadione except that methylene blue is a potent inhibitor of the choline esterase. 7. The mechanisms of the effects of menadione are discussed in relation to its ability to react with -SH groups, to its positive oxidation-reduction potential, and to its action on the aerobic glycolysis.

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Relation of Route of Administration to Toxicity of *dl*-Serine.*

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In a previous report,[1] we have described an injurious action of *dl*-serine when administered by stomach tube. In male rats on an experimental diet, this procedure resulted in severe clinical disturbances (anorexia, loss in weight, albuminuria), demonstrable pathological lesions in the kidney, and a high mortality. It was thought that a comparison of the effects of administering the amino acid by routes other than by stomach tube might throw some light on the mechanism of the toxic action of *dl*-serine.

Experimental. Male albino rats (Rockland) weighing 95-105 g were transferred from the stock diet to an experimental diet (Diet 4), containing "Labco" vitamin-free casein 10 parts, dextrin 37, sucrose 37, Crisco 5, cod liver oil 5, "Ruffex" 2, salt mixture (Osborne and Mendel) 4.† Water was supplied *ad libitum*. After 7 days had elapsed, the daily administration of *dl*-serine by stomach tube, by intraperitoneal injection, or by admixture in the diet was initiated and continued for 14 days. The animals were maintained afterwards for an additional week on the experimental diet. A control group of rats received water by stomach tube for the same length of time. Body weight was recorded 3 times a week and food consumption daily.

Results. Mortality is recorded in Table I and composite curves of the changes in body weight are presented in Fig. 1. Where fatalities occurred, two separate curves are shown, one for the animals dying (Curves IIA and IIIA) and one for those surviving (Curves IIB and IIIB). The data on food consumption have been omitted for the sake of brevity but the values paralleled the changes in body weight.

Control animals receiving water only by stomach tube (Group I) showed no visible ill-effects and they all survived. Thus, even in animals on a deficient diet, prolonged stomach tubing is not harmful. Both the slow growth and the failure to gain weight during the 4th week may be ascribed to the low protein content of the diet, as well as to the probable development of a B-vitamin deficiency.

In conformity with our previous results, in Group II (receiving serine by stomach tube), a sudden loss in weight and serious disturb-

* This work was aided by a grant from the John and Mary R. Markle Foundation. A preliminary report appeared in *Federation Proceedings*, March, 1944, **3**, 10.

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† This diet is identical to diet 1,[1] except that the small amounts of vitamin B complex were omitted.