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• Present address: Department of Chemistry, Kyushu University, Fukuoka, Japan.

▲ To whom inquiries should be directed.

Action of Ultrasonicated Epinephrine: Potentiation of Isometric Contraction in Isolated Papillary Muscle from Dog Quiescent Heart

ANWAR A. HAKIM

Abstract □ Epinephrine solutions were exposed to ultrasonic frequencies or to oxidation by passing oxygen through the solution until a pink color was formed. The effects of these epinephrine solutions were investigated on the physiological functions of heart papillary muscles and their contractile element, *i.e.*, actomyosin. When added to the bathing medium containing papillary muscle from beating rat hearts, the modified epinephrine produced a gradual increase in the maximum response of the muscle to electrical stimulation. If the incubating medium contained papillary muscle from quiescent dog heart, the muscle did not respond to electrical stimulation alone or in the presence of epinephrine. However, when added to the bathing medium, the sonified or the oxidized epinephrine caused initiation and potentiation of isometric contractions in response to electrical stimulation. The effects of the altered epinephrines on papillary muscles from both types of hearts were concentration and time dependent. When added with either the sonified or the oxidized epinephrine, a "serum protein" isolated from human serum intensified and stabilized the effects of both forms of epinephrine on the papillary muscle from rat or dog quiescent heart. When added to a standard actomyosin solution in imidazole–potassium chloride–ethyleneglycol tetraacetate salt buffer at pH 7.0, adenosine triphosphate caused precipitation of the contractile protein, which was assessed by the increase in absorbance and was measured spectrophotometrically at 660 nm. When the incubation mixture contained increments of the sonified or the oxidized epinephrine, the precipitating effect of adenosine triphosphate was not changed. But, if added with either modified epinephrine, the serum protein caused two- or fivefold increases in the precipitation of the contractile elements from solutions incubated in the presence of the oxidized or sonified epinephrine, respectively.

Keyphrases □ Epinephrine, ultrasonicated or oxidized—potentiation of isometric contraction in isolated heart papillary muscle □ Isometric contraction in isolated heart papillary muscle—potentiation by ultrasonicated or oxidized epinephrine □ Heart papillary muscle—potentiation of isometric contraction by ultrasonicated or oxidized epinephrine □ Ultrasonicated epinephrine—potentiation of isometric contraction in isolated heart papillary muscle

Epinephrine inotropy in cardiac muscle is characterized by two distinct alterations in the contractile process. In isolated tissue preparations (1) as well as *in situ* (2, 3), administration of epinephrine brings about an increase in systolic force or shortening, with an appreciable reduction in the duration of contraction (4).

In recent years, most studies on cardiac muscle have been performed on papillary muscle from beating cat or rat heart. When bathed or perfused with physiological buffer solution, isolated myocardial preparations spontaneously undergo contractile failure. Clark *et al.* (5) suggested that the addition of serum to the buffer solution stabilized these failing preparations. Addition of various plasma fractions or dialysates also delayed the onset of cardiac decay. This led Gabel *et al.* (6) to hypothesize that fatigue in isolated muscle preparations might also be influenced by the presence or absence of blood fractions. Other investigators (7, 8) showed that cardiac muscle maintained in plain buffer solutions lost the poststimulation potential, but it was restored by serum; they confirmed the presence of cardioactive principles in blood plasma.

The aim of the present studies is to demonstrate the initiation and potentiation of isometric contractions in electrically stimulated dog heart papillary muscle with epinephrine modified by exposure to gaseous cavitation produced by ultrasonic frequencies or by limited oxidation of the amine.

EXPERIMENTAL

Materials—Epinephrine solutions were transformed to a light-pink pigment, metaphrine, by two methods:

1. Solutions (10^{-6} *M*) of epinephrine in physiological saline were exposed to ultrasonic frequencies. A sonic oscillator[1] was employed at 20 w. at the machine maximum output of 10 kc., 0.78 amp. and 60 cycles. The solutions were sonified for 120 min. at 0–4° until the formation of metaphrines, which was assessed by the appearance of a light-pink color (9).

[1] Raytheon, model DF 101.

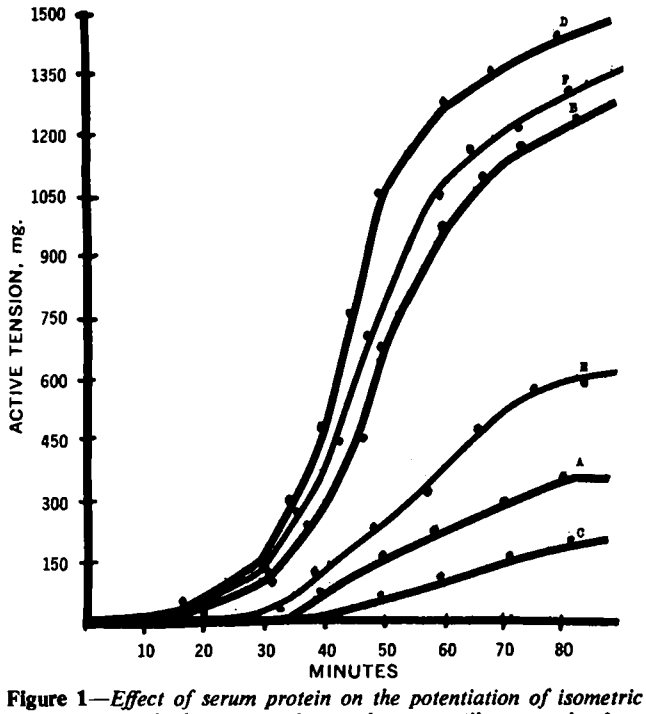

Figure 1—*Effect of serum protein on the potentiation of isometric contractions with ultrasonicated epinephrine: papillary muscles from quiescent dog heart incubated in the presence of 10^{-6} M epinephrine (A) and 10^{-6} M ultrasonicated epinephrine (B); tension developed by the papillary muscle incubating with 100 mcg. protein serum alone (C) and in the presence of 10^{-6} M ultrasonicated epinephrine (D); and tension generated in the muscle incubating with 10^{-6} M oxygenated epinephrine alone (E) and in the presence of 100 mcg. of the serum protein (F). The concentration of the ultrasonicated or oxidized epinephrine is taken as the concentration of epinephrine prior to treatment.*

2. A thin stream of oxygen was passed through solutions (10^{-6} *M*) of epinephrine in physiological saline to the appearance of a light-pink color, *i.e.*, 30 min. at 0–4°.

Dog hearts[2] were removed immediately after the animals expired and placed in cold preoxygenated bicarbonate–dextrose solution. Series of papillary muscles were removed, and the remaining tissue was frozen and saved for extraction of the contraction elements.

The papillary muscles were excised from the right ventricle and suspended from a force transducer[3] into a 50-ml. chamber containing 154.0 m*M* NaCl, 5.4 m*M* KCl, 7.4 m*M* $NaHCO_3$, 5.6 m*M* dextrose, and 5.6 m*M* $CaCl_2$ [*i.e.*, modified Krebs–Henseleit solution as described by Lefer (10)]. The papillary muscles were prepared with a preload of 1 g. and stimulated continuously at a frequency of 1/sec. for a duration of 3 msec. at 32 v. above threshold voltage. Under these experimental conditions, electrical stimulation alone did not generate isometric contractions in the papillary muscle from dog quiescent hearts. To the incubating medium containing the papillary muscle, aliquots of 0.05–0.20 ml. of 10^{-6} *M* ultrasonication modified epinephrine solution was added, and electrical stimulation was continued until maximum isometric contractions were generated at a steady state. The bathing medium was then drained, and the muscles were rinsed several times (5–10 times) with fresh buffer. Electrical stimulation was reinstated and continued to maximum steady state. Aliquots of the ultrasonication modified or the oxidized epinephrine, each alone or with the "serum protein," were then added into the incubating medium, and the isometric contractions were continuously recorded.

The human serum protein was prepared from freshly drawn blood as described by Hakim (11, 12).

Preparation of Human Heart Actomyosin—The hearts were obtained[4] from gunshot victims. The muscle was cleaned from adhering fat, minced, and then homogenized for 1 min. with three parts of Weber–Edsall solution (0.6 *M* KCl, 0.04 *M* NaCl, 0.04 *M* $NaHCO_3$, and 0.01 *M* Na_2CO_3). The homogenate was stirred continuously for 20 hr. at 4°, and the insoluble material was removed by centrifugation at $1000 \times g$ for 60 min. The pH of the supernate was adjusted to 7.0, and the actomyosin was precipitated with nine volumes of cold distilled water as described by Fanburg *et al.* (13).

Superprecipitation and clearing of actomyosin was followed by measuring the absorbance of an actomyosin suspension at 660 nm. in a spectrophotometer[5] after the addition of adenosine triphosphate. When added to an incubating mixture containing 0.1 *M* KCl, 0.5 m*M* imidazole, 0.1 m*M* ethylene glycol tetraacetate salt buffer at pH 7.0 and 0.50 mg. actomyosin (determined as protein), 0.2 m*M* adenosine triphosphate caused precipitation of the contractile protein, which was assessed by the increase in absorbance. When the incubating mixture contained aliquots of normal human serum or the isolated human serum protein, adenosine triphosphate caused an increase in the absorbance of the solution. The incubation mixtures were run in triplicate. The average deviation between the triplicate reactions was approximately 5.2%.

Determination of Adenosine Triphosphate, Phosphocreatine, and Lactic Acid—Immediately after the last contraction at the indicated time, the muscle was removed and quickly frozen in a dry ice–acetone mixture. An extract was then made in 0.25 *M* perchloric acid at 0°. All muscles were analyzed for adenosine triphosphate (14), phosphocreatine (15), and lactic acid (16).

RESULTS

Chemical Characterization of Modified Solutions of Epinephrine—When 1.0 ml. of either the ultrasonicated or the oxidized epinephrine was diluted with 4 ml. of pH 4.0 phthalate buffer and 1.0 ml. of 0.1 *N* iodine, followed by mixing for 5 min. and the addition of 2.0 ml. of 0.1 *N* sodium thiosulfate, a deep-red color was produced.

When 1.0 ml. of either modified epinephrine solution was diluted with 5 ml. of glacial acetic acid, followed by addition of 0.1 ml. of crystal violet test solution (0.5% solution) and titration with 0.1 *N* perchloric acid, 25.00 mg. of epinephrine, ultrasonicated epinephrine, or oxidized epinephrine consumed 1.36, 1.00, or 0.30 ml. of the acid, respectively.

When stored in the refrigerator for 3 months, epinephrine or its ultrasonicated form (10^{-6} *M* solutions in normal saline in 0.1 *N* HCl) remained clear with their initial specific optical rotation of −51 to −53°. Solutions of the oxidized form became brown in color with insoluble dark-brown sedimented particles.

These observations suggest that the ultrasonicated epinephrine is a metaphrine, while the oxidized epinephrine is mostly adrenochrome.

Physiological Effects on Rat Heart Papillary Muscle—When added to the bathing medium of rat heart papillary muscle obtained from beating heart, epinephrine at 10^{-6} *M* concentration produced a 15 ± 5.5% increase in the developed active tension and increased the rate of isometric contractions. Higher concentrations of epinephrine did not increase further the contractile tension but increased the rate of contraction to a magnitude that caused progressive decrease in the developed active tension where the muscle became refractive to electrical stimulation. The magnitude of the increase in the rate was concentration dependent.

If added to the bathing medium of papillary muscles obtained from beating hearts, ultrasonicated epinephrine solution at 10^{-6} *M* intensified the active tension without influencing the rate of the isometric contractions. The magnitude of the increase in tension was concentration and time dependent. Under the same experimental conditions, oxidized epinephrine at 10^{-6} *M* intensified the active tension without influencing the rate of the isometric contractions. The magnitude of the effect was also concentration and time dependent, equivalent to one-tenth the effect obtained with the ultrasonicated epinephrine.

Physiological Effects on Papillary Muscles from Dog Quiescent Hearts—Papillary muscles from quiescent dog hearts did not respond to electrical stimulation. Epinephrine or norepinephrine caused the muscle to respond to electrical stimulation after a latent period of 120–150 min. The magnitude of the response was time

[2] Obtained through Dr. L. Malasanos and Dr. S. Marotta of the Department of Physiology.
[3] TF .03.
[4] Cook County Morgue.
[5] Beckman model DU 2.

and concentration dependent. As shown by curves A in Figs. 1–3, an optimal concentration between 2 and 4 × 10^{-6} *M* produced a maximum tension of 575 ± 25 mg./mm.^{2}.

The data depicted by curves B of Figs. 1–3 suggest that, within 15 min., ultrasonicated epinephrine initiated and potentiated active tension in electrically stimulated dog heart papillary muscle. The magnitude of the developed active tension was concentration and time dependent. Electrical stimulation of papillary muscles incubating in the presence of 10^{-7} *M* ultrasonicated epinephrine for longer periods than 110 min. caused a progressive decrease in the developed active tension. These observations suggested that longer periods of electrical stimulation lead to the fatigue and decay of the papillary muscle.

Curves C of Figs. 1 and 2 indicate that 0.10 mg. of the serum protein prevented or delayed the fatigue or decay of the electrically stimulated papillary muscles.

Curve E of Fig. 1 suggests that, within 28–30 min., oxidized epinephrine initiated and potentiated the development of active tension in electrically stimulated dog heart papillary muscle. The magnitude of the effect was time and concentration dependent but equivalent to approximately one-third of the effect obtained with the ultrasonicated epinephrine. Curves F of Figs. 1 and 2 indicate that 0.10 mg. of the serum protein prevented or delayed the fatigue or decay of the electrically stimulated papillary muscle.

The results of experiments summarized in Fig. 1 demonstrate a lag period for the potentiation of isometric contractions in papillary muscle from quiescent dog heart with epinephrine, epinephrine derivatives, or the serum protein. Ultrasonicated epinephrine potentiated isometric contractions within 15 min. When combined with the ultrasonicated epinephrine, the serum protein enhanced and intensified the potentiation of the developed tension.

The data in Fig. 2 suggest that the maximum active tension potentiated with the ultrasonicated epinephrine decreased after 110 min. of continuous electrical stimulation. The presence of the serum protein prevented the effect of longer periods of electrical stimulation on the active tension of the dog heart papillary muscle. Therefore, the experiments suggest that the serum protein delays the fatigue and decay of the muscle and stabilizes its contractility and its response to electrical stimulation.

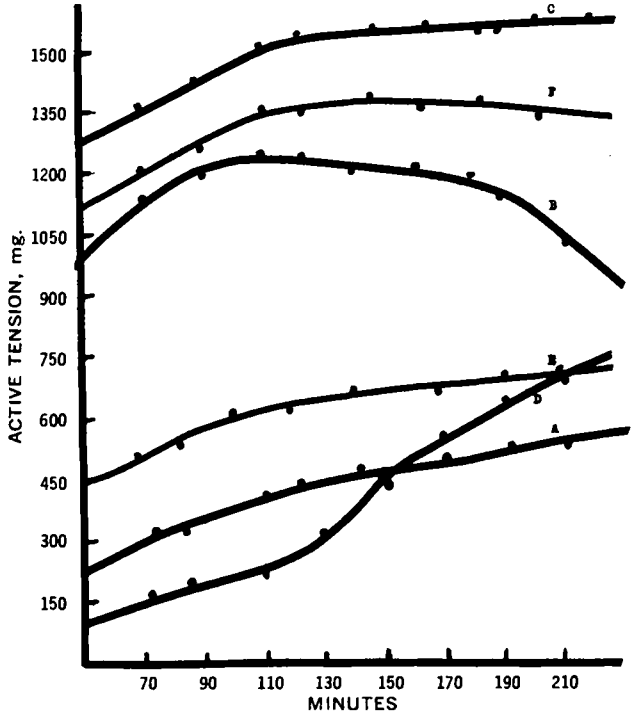

Figure 2—*Effect of serum protein on the decay of active contraction in papillary muscle from quiescent dog heart: the papillary muscles incubated with 10^{-6} M epinephrine (A) and with 10^{-8} M ultrasonicated epinephrine (B); developed tension with 100 mcg. of the serum protein alone (D) and in the presence of 10^{-8} M ultrasonicated epinephrine (C); and generated tension by muscles incubating with 10^{-8} M oxygenated epinephrine alone (E) and in the presence of 100 mcg. of the serum protein (F). The concentration of the modified forms of epinephrine is taken as the concentration of epinephrine prior to treatment.*

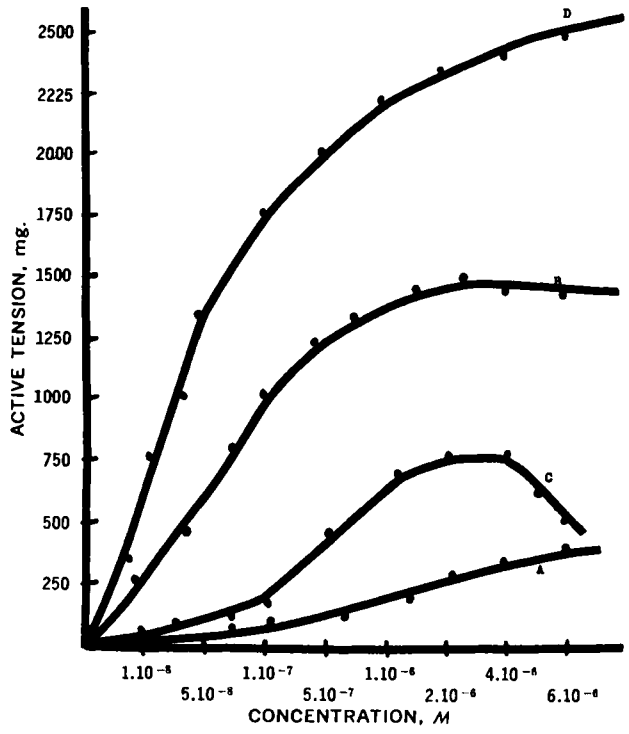

Figure 3—*Effect of concentration of the ultrasonicated epinephrine on the potentiation of active tension: papillary muscles from quiescent dog heart incubated with increasing concentration of epinephrine (A) or of ultrasonicated epinephrine (B); and tension developed by papillary muscles incubating with increasing concentrations of the serum protein (C) or with 100 mcg. of the serum protein and increasing concentrations of ultrasonicated epinephrine (D).*

The data in Fig. 3 suggest that the maximum tension developed by the papillary muscle in response to electrical stimulation depended on the concentration of epinephrine or its ultrasonicated or oxidized forms. An optimum concentration for epinephrine was found to be between 1 and 4 × 10^{-6} *M*. For the ultrasonicated or oxidized derivatives of epinephrine, as well as for the serum protein, the developed active tension was concentration dependent. There appears to be a certain relationship between concentration and effect in the action of the ultrasonicated epinephrine and the response to electrical stimulation. The combined effect of the serum protein and increasing concentrations of the ultrasonicated epinephrine was also dependent on the concentration of the derivative. Since the optical rotation of the ultrasonicated form was similar to that of epinephrine, the concentration of the ultrasonicated form was considered to be equivalent to the concentration of epinephrine prior to ultrasonication.

If added to the incubating medium containing papillary muscles from dog quiescent heart, the ultrasonicated epinephrine initiated isometric contraction in response to electrical stimulation. Tracings A, C, and E of Fig. 4 represent the typical response of papillary muscles from dog quiescent heart to electrical stimulation in the presence of ultrasonicated epinephrine. Tracings B and D show the effect of the epinephrine derivative on the developed active tension of the papillary muscle. In these two cases, the muscles were potentiated first with the ultrasonicated form to a maximum active tension; then the muscles were washed thoroughly with 5 l. of the oxygenated bicarbonate-dextrose solution and placed in fresh medium. The tracings suggest that the ultrasonicated epinephrine initiated and potentiated the development of active tension in the papillary muscle from dog quiescent heart. The magnitude of the potentiation and increase in the active tension induced by the ultrasonicated form is time dependent.

The tracings shown in Fig. 5 represent isometric contractions of papillary muscles from dog quiescent heart that was first potentiated

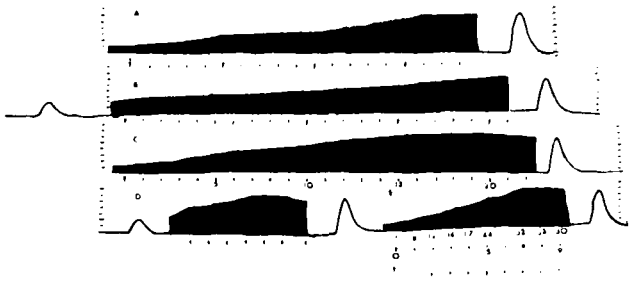

Figure 4—*Potentiation of active contraction of papillary muscle from quiescent dog heart. The arrow indicates addition of 0.05 ml. 10^{-6} M ultrasonicated epinephrine solution to electrically stimulated, nonresponding (patterns A, C, and E) and responding (B and D) papillary muscles of dog heart. The vertical scale is the tension of 36 mg./unit, and time (in minutes) is the horizontal scale. The top scale of tracing E indicates the tension potentiated with 0.10 ml. of ultrasonicated epinephrine solution in centigrams.*

to respond to electrical stimulation and then rinsed as described under *Experimental*. When the reinstated maximum active tension reached a steady state, aliquots of 50, 70, and 100 mcg. of the serum protein were added to the incubating medium. The tracings demonstrate the generation of a new maximum of active tension and suggest that the rate to attain this new maximum steady state depended on the concentration of the serum protein.

The tracings shown in Fig. 6 represent the isometric contractions of the papillary muscles from dog quiescent heart that was first activated to respond to electrical stimulation and then rinsed. When the reinstated maximum active tension reached a steady state, aliquots of 0.20 and 0.40 ml. of 10^{-6} *M* ultrasonicated epinephrine were added to the incubating medium. These tracings demonstrate that the rate of muscular decay or fatigue is faster at the higher concentration levels of the ultrasonicated epinephrine. If combined with the ultrasonified epinephrine, the serum protein delayed and reduced the magnitude of the decay caused by electrical stimulation (tracing C). The tracings indicate that the serum protein changed the mechanics of the contraction and produced an increase in the rate to peak tension and in the contraction–relaxation time. These observations suggest the role of the serum protein in the coupling excitation–contraction cycle of the contractile elements of the cardiac muscle.

The data in Fig. 7 summarize the ultrasonicated epinephrine concentration response alone and in the presence of three concentrations of the serum protein. Again, the concentration of the ultrasonicated form was taken as equivalent to the initial concentration of epinephrine prior to ultrasonication. The results suggest a linear relationship for the ultrasonified epinephrine between 10^{-9} and

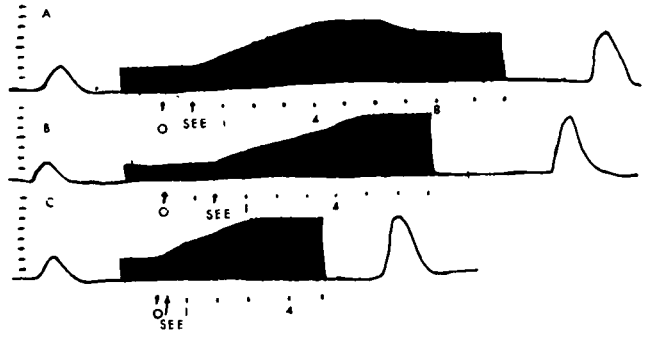

Figure 5—*Effect of concentration of the serum protein on the developed active tension. Papillary muscle from quiescent dog heart was first potentiated to respond to electrical stimulation with the ultrasonicated modified epinephrine and then rinsed as described in the text. At the reinstated maximum active tension at the steady state, aliquots of 50, 70, and 100 mcg. (tracings A, B, and C, respectively) of the serum protein were added to the incubating medium. The vertical scale is the tension of 36 mg./unit, and time (in minutes) is the horizontal scale.*

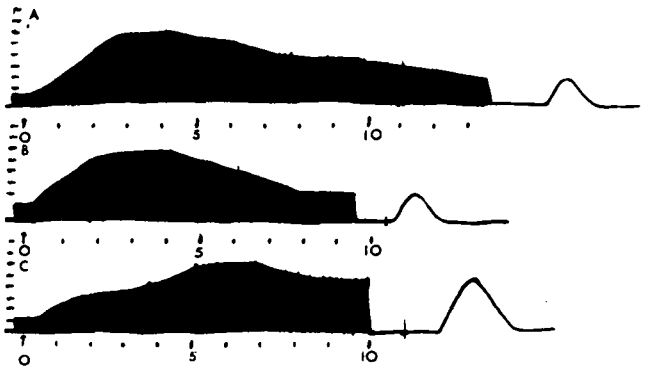

Figure 6—*Potentiation of active contraction with 0.20 and 0.40 ml. of 10^{-6} M ultrasonicated epinephrine alone (tracings A and B, respectively) and 0.40 ml. of 10^{-6} M ultrasonicated epinephrine with 100 mcg. of the serum protein together (tracing C).*

5×10^{-7} *M* concentration and the generated active tension in the rat heart papillary muscle. These experiments indicate that the serum protein enhanced the effect of the ultrasonicated epinephrine in the papillary muscle model. The magnitude of the enhancing effect is dependent on the employed serum protein concentration.

Effect of Epinephrine, Ultrasonicated Epinephrine, Oxidized Epinephrine, and Serum Protein on Superprecipitation of Human Heart Actomyosin—When added to a standard incubating mixture containing 0.50 mg. of actomyosin from human heart, adenosine triphosphate caused precipitation of the contractile protein, described as the superprecipitation of the contractile protein, and was assessed by the increase in absorbance of the solution (curve A, Fig. 8).

The human heart actomyosin preparation responded well to

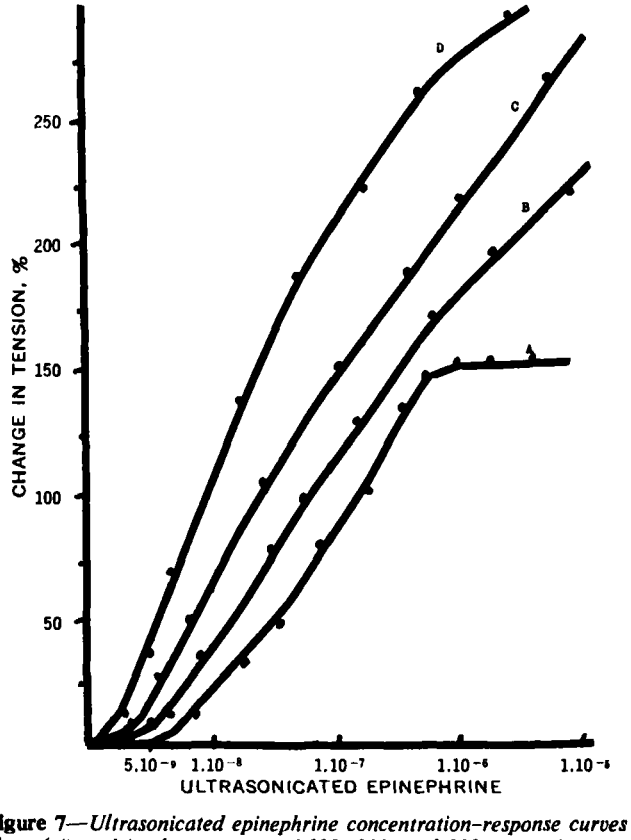

Figure 7—*Ultrasonicated epinephrine concentration–response curves alone (A) and in the presence of 100, 200, and 300 mcg. of serum protein (B, C, and D, respectively) in rat heart papillary muscles. Each point is the average of four muscles with an average deviation of ±5.3. Isometric tension force of the rat heart papillary muscle before the ultrasonicated epinephrine = control, 3.8 ± 0.8 g./mm.².*

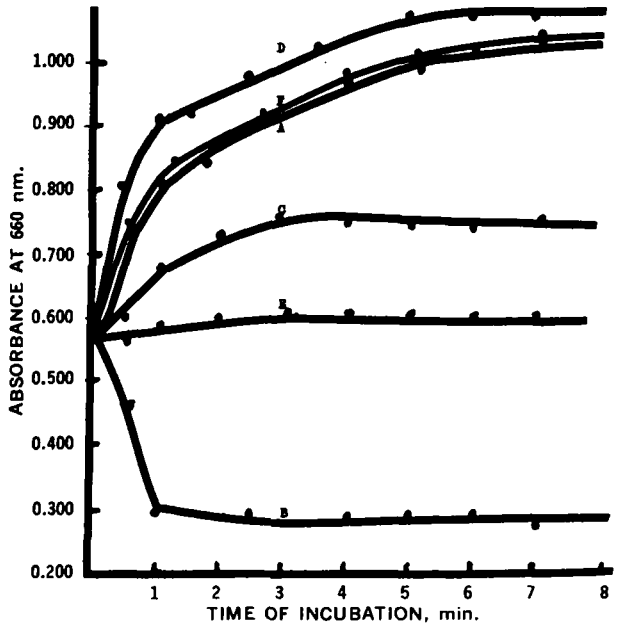

Figure 8—*Effect of ultrasonicated and oxidized epinephrine on actomyosin superprecipitation. The incubation mixture contained: 5.0 mM $MgCl_2$, 5.0 mM adenosine triphosphate, 20 mM tromethamine buffer of pH 7.5, 0.05 mM KCl, and 0.50 mg. actomyosin/ml. (A); all of A with 0.1 mM ethylene glycol tetraacetic acid (B); all of A with ultrasonicated epinephrine, 10^{-6} M (C), 10^{-3} M (D); all of A with epinephrine, 10^{-3} M (E); or all of A with oxidized epinephrine, 10^{-3} M (F).*

ethylene glycol tetraacetic acid, a chelating agent (Fig. 8). Removal of calcium from the medium with ethylene glycol tetraacetic acid resulted in the reversal of superprecipitation and clearing of the actomyosin suspension, which was followed by the decrease in absorbance (curve B). Addition of small amounts of the ultrasonicated epinephrine, 10^{-6} or 10^{-3} *M*, caused reversal of the clearing effect of the ethylene glycol tetraacetic acid. When added to the ethylene glycol tetraacetic acid, epinephrine at 10^{-3} *M* inhibited both the precipitation effect of adenosine triphosphate and the clearing effect of the chelating agent.

The data depicted by curves C and D suggest a concentration-dependent response for the ultrasonicated epinephrine. The action differed from that of epinephrine in its intensifying and enhancing effects on the superprecipitation of the actomyosin.

Figure 9 shows the effect of the ultrasonicated epinephrine alone (curve B), with the serum protein (curve C), and with the heat-denatured serum protein (curve D). In contrast to the effect of the serum protein, the heat-denatured serum protein did not influence the effect of the ultrasonicated epinephrine.

Effect of Ultrasonicated and Oxidized Epinephrine on Concentration of Adenosine Triphosphate, Phosphocreatine, and Lactic Acid of Dog Heart Papillary Muscle—Extracts of nonresponding and responding papillary muscles were prepared at time intervals during the potentiation period and analyzed for adenosine triphosphate, phosphocreatine, and lactic acid. The data (Table I) revealed no change in the concentration of adenosine triphosphate, phosphocreatine, or lactic acid in muscle which did not respond to electrical stimulation. When the ultrasonicated or oxidized epinephrine was added to the incubation medium, the initiation and potentiation of isometric contractions in response to electrical stimulation were accompanied by a progressive increase in adenosine triphosphate, phosphocreatine, and lactic acid (micromoles per gram of fresh weight) of the muscle. These experiments suggest that the amount of chemical energy released from the high energy phosphate compounds was utilized during the generation of active tension. The data also indicate that the ultrasonicated epinephrine stimulated the conversion of chemical energy in the endogenous adenosine triphosphate and phosphocreatine to the contractile, *i.e.*, mechanical, energy. When combined with the ultrasonicated epinephrine, the serum protein maintained the adenosine triphosphate and phosphocreatine at levels of 280 ± 10.5 and 350 ± 15 μmoles/gram weight, respectively[6]. These experiments suggest that the major functions of the serum protein are in the adenosine triphosphate regeneration systems and in maintaining the high energy phosphate compounds at a concentration capable of supporting development of active tension in muscular contraction.

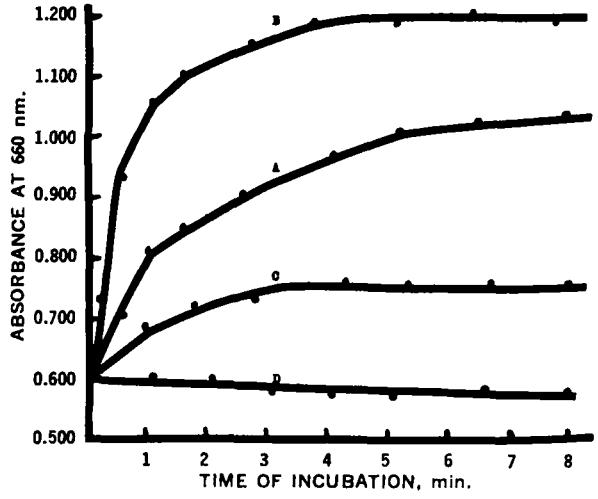

Figure 9—*Effect of ultrasonicated epinephrine and of the serum protein on actomyosin superprecipitation. The incubation mixture contained: 5.0 mM $MgCl_2$, 5.0 mM adenosine triphosphate, 20.0 mM tromethamine buffer of pH 7.5, 0.05 mM KCl, and 0.5 mg. of actomyosin/ml. (A); all of A with 10^{-6} M ultrasonicated epinephrine with 100 mcg. of the serum protein (B); all of A with 10^{-6} M ultrasonicated epinephrine, 0.1 mM ethylene glycol tetraacetic acid with 100 mcg. of the serum protein (C); all of A with 100 mcg. of the 80° denatured serum protein, 10^{-6} M ultrasonicated epinephrine with ethylene glycol tetraacetic acid (D).*

DISCUSSION

The effect of epinephrine on the cardiac muscle is well known (1–4). Epinephrine slightly increased the active tension, *i.e.*, developed tension of the muscle, and the rate of cardiac muscle, *i.e.*, the number of heart beats. The lag period required for the potentiation and initiation of active contraction in the presence of epinephrine or norepinephrine supports the suggestion that the effect of these amines on the developed tension of the papillary muscle is the result of the action of a modified form of the amine. Possible modification is the oxidation of epinephrine by the stream of oxygen gas employed to supply oxygen to the muscle and to stir the incubation medium surrounding the papillary muscle.

The superprecipitation of natural actomyosin was accelerated by a minute amount of calcium-removing substance, such as chelating agents, or the relaxing factor (17, 18). In view of the importance of this phenomenon in the physiological events of muscle contraction (19), the effect of epinephrine and of its sonified and oxidized forms, alone and in presence of the serum protein, was studied on human cardiac actomyosin. Paddle and Hauggard (20) found that epinephrine failed to initiate contraction in the arrested heart; their measurements of lactate production, glucose 6-phosphate content, and phosphorylase "a" activity showed that epinephrine also had no effect on glycolysis. Norepinephrine also had no effect on the papillary muscles from dog or human quiescent hearts[6]. The tracings of the initiation and potentiation of isometric contractions, with the enhancing effects on the precipitation of the purified actomyosin, suggest that ultrasonicated epinephrine, alone or in presence of the serum protein, stimulates the *in vivo* contractile elements to initiate the development of isometric contractions.

Current concepts of excitation-contraction coupling suggest that procedures that increase the sarcotubular calcium pool augment myocardial contractility by increasing the amount of calcium released at the actomyosin junction following membrane depolarization resulting from electrical stimulation (21, 22) and, conversely,

[6] Unpublished data.

Table I—Concentration of Adenosine Triphosphate, Phosphocreatine, and Lactic Acid in Papillary Muscle from Quiescent Dog Heart

Muscle	Minutes	Adenosine Triphosphate, mcg./Gram Weight	Phosphocreatine, mcg./Gram Weight	Lactic Acid, mcg./Gram Weight
Nonresponding	0	4.87 (5)[a] 2.50 to 7.24	14.67 (4) 10.55 to 18.41	1.63 (5) 0.95 to 2.31
	15	4.95 (6) 4.05 to 5.85	14.50 (4) 12.25 to 16.75	1.62 (4) 1.05 to 2.15
	30	5.15 (5) 4.25 to 6.05	14.25 (5) 12.15 to 16.35	1.75 (5) 0.95 to 2.55
	45	4.92 (4) 4.25 to 5.55	14.60 (5) 13.10 to 16.20	1.68 (5) 1.15 to 2.21
	60	4.85 (5) 4.05 to 5.65	14.75 (4) 12.25 to 17.25	1.65 (4) 1.15 to 2.15
	120	5.05 (5) 4.60 to 5.50	15.15 (5) 12.75 to 17.55	1.76 (4) 1.15 to 2.37
Responding (ultrasonicated epinephrine)	0	5.65 (6) 4.25 to 7.05	15.75 (5) 14.25 to 17.25	7.68 (5) 4.60 to 10.76
	15	77.25 (5) 70.25 to 84.15	82.15 (4) 68.50 to 95.80	16.75 (5) 11.50 to 22.00
	30	110.75 (5) 92.15 to 119.65	136.20 (4) 102.50 to 169.65	24.50 (6) 20.75 to 23.50
	45	177.10 (4) 150.5 to 204.3	205.25 (5) 165.4 to 255.6	90.25 (5) 65.25 to 115.25
	60	231.25 (5) 215.2 to 247.40	264.50 (4) 215.25 to 323.75	190.75 (5) 110.25 to 270.25
Responding (oxygenated epinephrine)	0	5.60 (4) 4.15 to 7.05	15.15 (4) 13.80 to 16.50	5.60 (5) 4.12 to 7.08
	15	55.35 (4) 40.75 to 69.95	64.35 (4) 53.25 to 75.45	13.20 (4) 12.15 to 14.25
	30	85.75 (4) 73.15 to 98.35	105.45 (4) 98.75 to 112.15	19.75 (4) 18.25 to 21.25
	45	126.35 (4) 124.50 to 128.20	167.25 (4) 165.45 to 169.05	57.65 (4) 56.25 to 59.05
	60	185.35 (4) 184.25 to 186.45	205.45 (4) 202.75 to 208.15	108.25 (4) 106.95 to 109.55

[a] Number of experiments is indicated in parentheses.

methods that decrease the calcium pool diminish contractility (23).

When a muscle is stimulated electrically, chemical reactions occur that result in activation of the contractile elements and constitute the active state of the muscle. Alone, electrical stimulation of papillary muscle from dog or human heart did not potentiate or initiate isometric contractions and did not cause changes in the concentration of adenosine triphosphate, phosphocreatine, or lactic acid. Therefore, in these muscles, electrical stimulation did not activate the contractile elements. In the presence of ultrasonicated or oxidized epinephrine in the incubating medium, electrical stimulation of the papillary muscle from quiescent hearts did potentiate isometric contractions, with a progressive increase in the concentration of adenosine triphosphate, phosphocreatine, and lactic acid. The magnitude of the developed active tension of the isometric contractions and the magnitude of the increase in adenosine triphosphate, phosphocreatine, and lactic acid concentrations were time dependent. These observations suggest that the presence of the modified forms of epinephrine caused the activation of certain metabolic processes, which involve the high energy phosphate compounds adenosine triphosphate and phosphocreatine, with the conversion of chemical energy to the contractile element actomyosin. When combined with ultrasonicated or oxidized epinephrine, the serum protein stabilized the initiation and potentiation of the developed tension. These observations are in agreement with the mechanism of action of the serum protein reported earlier (11, 12) and with the effect of serum on the fatigue and decay of normal muscles (5–8).

Preliminary chemical studies suggest similarities in the structure of the ultrasonicated and oxidized forms of epinephrine prepared as described in the *Experimental* section. The physiological effects on the papillary muscles from dog quiescent hearts also indicate similarities between these two modified forms of epinephrine. Difference in the stability of solutions containing the modified forms of epinephrine indicate certain differences in the identity of oxidized and ultrasonicated epinephrine.

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Adsorption of Nonionic Surfactants on Sulfathiazole and Naphthalene and Flocculation–Deflocculation Behavior of These Suspensions

A. OTSUKA▲, H. SUNADA, and Y. YONEZAWA

Abstract □ The adsorption of polyoxyethylated nonyl-phenols and octyl-phenols, having different polyoxyethylene chain lengths, on sulfathiazole and naphthalene was studied. Adsorption isotherms for these surfactants on sulfathiazole are S-shaped, and the adsorption amounts at saturation were greater than those for the closest packing at the air–solution interface. These results suggest that some multilayer adsorption or aggregate formation on the surface of solid occurred above a certain concentration. In the case of naphthalene, all isotherms are of the Langmuir type and the areas per molecule calculated from the saturation adsorption were found to be considerably larger than the values for the air–solution interface. It is likely that the hydrocarbon chains adhere to the surface of naphthalene, lying flat, while the polyoxyethylene chains are directed toward the solution. Flocculation–deflocculation measurements for both systems were carried out by the sedimentation volume method, and the results support the adsorption mechanisms described.

Keyphrases □ Polyoxyethylated octyl- and nonyl-phenols—adsorption on sulfathiazole and naphthalene, flocculation–deflocculation behavior of suspensions □ Surfactants, nonionic—adsorption on sulfathiazole and naphthalene, flocculation–deflocculation behavior of suspensions □ Adsorption of polyoxyethylated octyl- and nonyl-phenols on sulfathiazole and naphthalene—adsorption isotherms, flocculation–deflocculation behavior □ Sulfathiazole—adsorption behavior of polyoxyethylated octyl- and nonyl-phenols □ Naphthalene—adsorption behavior of polyoxyethylated octyl- and nonyl-phenols □ Flocculation–deflocculation behavior—suspensions of polyoxyethylated octyl- or nonyl-phenols and sulfathiazole or naphthalene

Many surfactants are capable of affecting the state of dispersion of powder particles in suspension. To examine the mechanism of action of surfactants in flocculation–deflocculation phenomena, it is desirable to investigate the adsorption of surfactants on powders. Several workers have studied the adsorption behavior and/or the dispersing action of nonionic surfactants. The adsorption of polyoxyethylated alkyl-phenols on sand and of ethylene oxide–propylene oxide condensates on quartz has been reported (1, 2). Kuno and Abe (3) reported the adsorption of polyoxyethylated nonyl-phenols on calcium carbonate and carbon black from both aqueous and cyclohexane solutions. The adsorption of nonionic surfactants by textile fibers was studied by Weatherburn and Bayley (4), Nemoto and Miwa (5), and Schott (6).

Corkill *et al.* (7) studied the adsorption of polyoxyethylene glycol monoalkyl ethers on graphon at different temperatures and obtained the adsorption isotherms. They also measured the heats of adsorption calorimetrically. Other investigators (8, 9) commented on the influence of the adsorption of polyoxyethylene glycol monoalkyl ethers on the stability of silver iodide and polystyrene latex sols. In most of these studies, the adsorbents examined were inorganic compounds or polymers. Only a few workers have performed this kind of study using pharmaceutical or organic powders. The adsorption of polyoxyethylated nonyl-phenol on sulfamines (10) and of several nonionic surfactants on griseofulvin (11) has been reported.

The purpose of this work was to investigate the adsorption of nonionic surfactants on some organic powders and to find a certain relation between the flocculation–deflocculation behavior of these suspensions and the adsorption results. The surfactants used were polyoxyethylated nonyl-phenols and octyl-phenols. Sulfathiazole (polar compound) and naphthalene (nonpolar) were selected as adsorbents.

EXPERIMENTAL

Materials—Series of polyoxyethylated nonyl-phenols and octyl-phenols were prepared by repeated distillation of commercial products[1]. The average polyoxyethylene chain lengths were determined from their hydroxyl values. Table I gives the CMC and

[1] Nippon Nyukazai Co. Ltd., Tokyo, Japan.