Thermal Injury: Action of Acute Burn Serum on Rat Heart Papillary Muscles (37591)

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Over the last few years, data were obtained which suggest the release by thermal injury of an inhibitor of adenosine 5′-triphosphate (ATP)-generated tension (1). A "toxic glycoprotein" isolated from *in vitro* scalded human skin depressed the generation of ATP-generated tension in glycerol-extracted muscle fibers (2–4).

In view of the many similarities in the physicochemical and quantitative enzymatic properties of cardiac and skeletal contractile proteins (5, 6) a basically similar mechanism for the regulation of contraction and relaxation could be expected to operate in these two muscles. The release by thermal injury into the circulation of physiologically active tissue breakdown products, which act as toxic antigens could cause disturbances in control mechanisms leading to an impaired regulation of myocardial contractility.

In the present investigations, the effects of serum from thermally injured patients were studied on the developed tension in rat heart papillary muscle contracting in response to electrical stimulation.

Methods. Blood samples were obtained from Summer L. Koch burn ward at Cook County Hospital. The blood was allowed to clot at room temperature, and the serum was separated by centrifugation at 1000*g* for 15 min. The serum was dialyzed against 30-fold volumes and three changes of the bicarbonate dextrose solution, then frozen in multiple aliquots for later analysis.

The papillary muscles were excised from the right ventricle of the beating rat heart. The muscles were suspended from a TF 0.03 force transducer, at 1 g preload, into a 50 ml chamber containing 154.0 m*M* NaCl, 5.4 m*M* KCl, 7.1 m*M* $NaHCO_3$, 5.6 m*M* glucose and 5.6 m*M* $CaCl_2$ as described by Lefer (7). The papillary muscles were then stimulated by a square wave of 1 msec duration 1/sec at 32 V above threshold.

The data from preliminary control experiments, where increments of physiological saline, or dialyzed serum from normal volunteers were added to the incubating medium containing papillary muscles contracting in response to electrical stimulation, indicated that the magnitude of the developed tension varied from one muscle to another depending on the length of the muscle. Therefore the muscles used measured 5 to 10 mm in length with a cross-sectional area, calculated on the basis of weight, assuming the muscle to be cylindrical (8), of 0.5 to 1.0 mm^2. In all the reported experiments, isometric contractions were performed at the apex of the length–active tension curve, L_{max}. Each preparation was allowed to stabilize for 120 min before the serum was introduced into the bathing medium.

Results. When added to the incubating bicarbonate dextrose medium containing papillary muscles contracting at a steady state in response to electrical stimulation, increments of dialyzed normal serum, increased the developed tension. The magnitude of the increase was time and concentration dependent. During a 12 min period, 0.10 ml of the dialyzed serum caused approximately 3% increase in the developed tension. In 25 blood samples, 0.10 ml of normal serum caused an immediate slight decrease, which was then followed by a progressive increase in the developed tension (9, 10).

The data presented in Table I show the

TABLE I. Effect of Serum from Thermally Injured Patients on Developed Tension in Rat Heart Papillary Muscles.[a]

Thermal injury		Days after injury	Developed tension (g/mm²) Control time (min)			
Patient	Degree[b]		0	1	6	12
Normal	—	—	5.15 ± 0.28	4.90 ± 0.22	5.25 ± 0.29	5.45 ± 0.30
E.R.	15, 40	6	4.80 ± 0.20	4.16 ± 0.18	2.08 ± 0.09	1.44 ± 0.05
J.Ne.	18, 39	16	5.40 ± 0.28	4.62 ± 0.18	3.19 ± 0.14	2.09 ± 0.09
A.O.	0, 43	4	5.76 ± 0.30	4.80 ± 0.20	3.96 ± 0.15	1.60 ± 0.06
L.Tr.	4, 32	3	5.44 ± 0.28	4.67 ± 0.18	4.16 ± 0.18	2.11 ± 0.10
H.C.	0, 56	5	5.82 ± 0.30	5.12 ± 0.17	4.05 ± 0.17	1.56 ± 0.08
D.S.	45, 80	0	5.84 ± 0.32	5.38 ± 0.28	4.15 ± 0.17	1.25 ± 0.05
R.S.	0, 29	6	6.08 ± 0.36	6.35 ± 0.40	5.66 ± 0.30	4.75 ± 0.20
E.A.	10, 21	6	6.29 ± 0.37	5.93 ± 0.32	4.59 ± 0.29	2.23 ± 0.11
A.N.	12, 68	2	6.23 ± 0.37	5.66 ± 0.30	5.00 ± 0.27	2.31 ± 0.12
D.H.	10, 44	6	5.90 ± 0.36	5.54 ± 0.34	4.50 ± 0.19	1.98 ± 0.10
F.C.	5, 70	3	6.72 ± 0.45	5.72 ± 0.29	2.84 ± 0.15	2.66 ± 0.08
D.Sp.	19, 36	6	6.07 ± 0.36	5.58 ± 0.29	4.32 ± 0.27	2.23 ± 0.07
A.J.	30, 65	3	5.66 ± 0.30	4.49 ± 0.17	2.94 ± 0.19	2.52 ± 0.08
M.T.	22, 50	0	4.99 ± 0.20	3.10 ± 0.14	2.26 ± 0.11	1.38 ± 0.04
I.M.	14, 47	11	6.38 ± 0.38	5.73 ± 0.29	4.85 ± 0.21	2.78 ± 0.09
C.K.	5, 70	23	6.58 ± 0.40	4.90 ± 0.22	3.67 ± 0.28	1.98 ± 0.10
Mean av			5.23 ± 0.25	5.13 ± 0.24	3.88 ± 0.19	2.18 ± 0.15

[a] Aliquots of 0.10 ml of the dialyzed serum were used. Each serum from the acute burn injury was assayed on three separate muscles, and the standard deviation is shown as (±).

[b] Degree of thermal injury is reported as the percentage of full thickness injured (first number), and as percentage of total body area injured (second number).

effect of 0.10 ml of dialyzed serum from acute thermally injured patients on the developed tension (g/mm²) in rat papillary muscle contracting in response to electrical stimulation. The data indicate that serum from acute thermally injured patients depresses the developed tension.

The extent of inhibition caused by blood samples drawn within the first 7 days of injury varied from one patient to another. If blood samples were drawn from the same patient at weekly intervals, the inhibitory effect decreased from 1 wk to another. The serum from severe fatally injured patients with 85% or greater total body injury depressed immediately the response produced by the standard stimulus. In these cases, high voltage, *i.e.*, 70 V with longer duration per stimulus, *i.e.*, 30–50 msec were needed to obtain a response. The sera of these patients were not included in the data summarized in the present studies, but were used to isolate the "acute burn serum inhibitor" (15).

Neutralization of the inhibitory effects of the acute burn with serum from convalescent thermally injured subjects is shown in Fig. 1. The top tracing A in Fig. 1, shows a typical effect of 0.05 ml of the dialyzed serum from acute thermally injured patient D.H. with 44% total body injury. When mixed and incubated for 5 min at 37° with an equal volume of the dialyzed serum from convalescent patients, D.S., or E.A., the acute burn serum produced the effects shown by the tracings B and D, respectively. The tracings suggest partial and complete neutralization of the inhibitory effects of the acute burn serum, respectively. When mixed with the dialyzed acute burn serum, an equal volume of dialyzed normal serum, either had no effect or increased the inhibitory effects of the acute burn serum. The last tracing of Fig. 1, suggests an increase in the inhibitory effect.

Neutralization of the depressing effect of a series of sera from acute with sera from

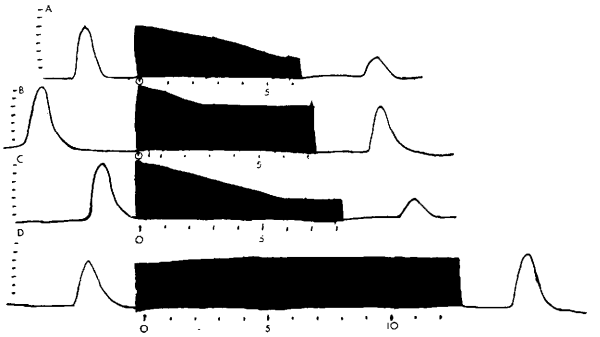

FIG. 1. Neutralization of the effect of serum from acute with serum of convalescing thermally injured patients: Aliquots of 0.25 ml of the dialyzed acute serum D.H. (drawn 6 days after injury) was mixed with: (A) 0.25 ml of physiological saline; (B) 0.25 ml of dialyzed serum from convalescing patient D.S. (drawn 7 wk after injury); (C) 0.25 ml of dialyzed normal human serum; (D) 0.25 ml of dialyzed serum from convalescing patient E.A. (drawn 9 wk after injury). The mixtures were incubated for 5 min at 37°, then 0.10 ml was added to the bathing medium containing rat papillary muscle contracting at a steady state to electrical stimulation. The developed tension 1.0 g/mm^2 per unit of the vertical scale, and the time (min) on the horizonal scale.

convalescent thermally injured subjects is indicated by the data summarized in Table II. If combined, equal volumes of the dialyzed acute burn with normal serum depressed, but equal volumes of acute burn and convalescent burn serum had no effect on the developed tension of rat papillary muscles. The extent of the depressing effect depended on the inclusion of both the serum from acute and the serum of convalescent thermal injury.

Discussion. The present studies showed that dialyzed serum from normal volunteers slightly increased, and serum from acute thermally injured patients, depressed the developed tension of rat papillary muscles contracting in response to electrical stimulation. Studies on the physiological effects of calf and normal human serum revealed the presence of a serum protein, which enhanced adenosine 5′-triphosphate (ATP)-generated tension in glycerol-extracted psoas muscle fibers, and increased the developed tension of rat and dog papillary muscle (9, 10) contracting in response to electrical stimulation. Earlier investigations suggested the release by thermal injury of an inhibitor of adenosine 5′-triphosphate (ATP)-mediated tension in the glycerol-treated muscle fibers (1, 14).

Severe experimental burns in the dog caused a decrease in cardiac output with an impairment of myocardial contractility (11–13). Alterations in cardiac function also, have been suggested from electrocardiographic tracings of acute thermally injured patients (14). In an attempt to determine to what extent the information gained about the effect of acute thermally injured patients and development of ATP-generated tension in glycerinated fibers applies to the myocardium, most of our studies have been performed on rat papillary muscles. These papillary muscles were obtained from beating hearts to preserve the natural integrity of the contractile elements. Both the depression of developed tension of rat papillary muscle contracting isometrically in response to electrical stimulation, and the decrease in cardiac output caused by the serum of acute thermally injured patients and by severe burn, suggest primary myocardial injury (13).

The serum of severely, or fatally thermally injured patients, caused a decrease in the voltage threshold required by the papillary muscle to respond to electrical stimulation. This observation suggests that the serum from acute thermally injured patients also influences the membrane physiological functions.

TABLE II. Neutralization of the Inhibitory Activity of Serum from Acute with Serum from Convalescent Thermally Injured Patients.[a]

	Developed tension (g/mm²)				
	Control time (min)				
Serum mixed (1:1)	0	1	6	12	15
Normal saline : Normal serum[b]	5.56 ± 0.32	5.76 ± 0.33	5.85 ± 0.33	5.97 ± 0.34	6.39 ± 0.35
I. Normal saline : convalescent					
: A.N. (5 wk)[c]	6.80 ± 0.45	6.75 ± 0.46	6.80 ± 0.38	6.77 ± 0.45	6.75 ± 0.43
: E.R. (8 wk)	6.02 ± 0.39	5.61 ± 0.32	5.81 ± 0.33	5.74 ± 0.33	5.57 ± 0.32
: J.Ne. (6 wk)	5.84 ± 0.35	5.44 ± 0.32	5.52 ± 0.31	5.82 ± 0.36	6.25 ± 0.54
: L.Tr. (6 wk)	5.60 ± 0.32	4.20 ± 0.19	4.80 ± 0.19	5.20 ± 0.20	5.80 ± 0.23
: H.C. (7 wk)	6.06 ± 0.36	5.22 ± 0.27	5.43 ± 0.25	5.58 ± 0.25	5.65 ± 0.25
: D.S. (10 wk)	6.80 ± 0.46	5.84 ± 0.35	5.52 ± 0.31	5.65 ± 0.30	5.70 ± 0.32
: D.Sp. (12 wk)	6.20 ± 0.38	6.60 ± 0.40	6.82 ± 0.42	6.93 ± 0.45	6.95 ± 0.45
Mean average	6.20 ± 0.32	5.66 ± 0.28	5.82 ± 0.29	5.95 ± 0.29	6.09 ± 0.30
II. Normal serum : convalescent serum					
: A.N. (5 wk)	6.90 ± 0.45	6.85 ± 0.45	6.95 ± 0.47	7.05 ± 0.50	7.25 ± 0.50
: H.C. (7 wk)	6.26 ± 0.37	6.05 ± 0.35	6.25 ± 0.37	6.50 ± 0.40	6.65 ± 0.45
: E.R. (8 wk)	6.15 ± 0.40	5.95 ± 0.37	6.25 ± 0.40	6.45 ± 0.45	6.50 ± 0.50
: J.N. (7 wk)	5.75 ± 0.30	5.45 ± 0.28	5.70 ± 0.30	5.80 ± 0.36	5.95 ± 0.39
: D.S. (10 wk)	6.75 ± 0.45	6.25 ± 0.43	6.70 ± 0.45	6.85 ± 0.47	6.90 ± 0.50
Mean average	6.36 ± 0.32	6.07 ± 0.30	6.37 ± 0.31	6.53 ± 0.32	6.66 ± 0.33
III. Normal serum : acute burn serum					
: D.S. (1 day)	5.95 ± 0.29	5.18 ± 0.25	3.55 ± 0.17	1.05 ± 0.05	0.95 ± 0.04
: A.J. (3 days)	6.05 ± 0.30	4.95 ± 0.24	2.75 ± 0.13	1.15 ± 0.05	0.90 ± 0.04
: D.Sp. (6 days)	6.15 ± 0.30	5.75 ± 0.28	4.25 ± 0.21	2.55 ± 0.12	2.25 ± 0.11
: M.T. (2 days)	5.25 ± 0.26	3.15 ± 0.15	2.34 ± 0.11	2.03 ± 0.10	1.38 ± 0.06
: H.C. (5 days)	5.95 ± 0.29	5.19 ± 0.25	4.15 ± 0.20	1.50 ± 0.07	1.05 ± 0.05
Mean average	5.87 ± 0.24	4.84 ± 0.24	3.41 ± 0.17	1.65 ± 0.08	1.30 ± 0.06
IV. Acute burn serum : convalescent serum					
D.S. (1 day) : E.R. (8 wk)	5.82 ± 0.30	6.12 ± 0.36	5.76 ± 0.29	5.64 ± 0.32	5.55 ± 0.31
A.J. (3 days) : D.S. (10 wk)	6.03 ± 0.39	5.49 ± 0.30	6.40 ± 0.38	6.84 ± 0.46	6.10 ± 0.37
D.Sp. (6 days) : D.S. (10 wk)	5.50 ± 0.32	4.61 ± 0.30	5.28 ± 0.26	5.68 ± 0.27	5.88 ± 0.39
M.T. (2 days) : D.S. (10 wk)	6.01 ± 0.39	5.80 ± 0.33	5.78 ± 0.33	5.96 ± 0.35	6.15 ± 0.40
A.J. (3 days) : E.A. (9 wk)	5.21 ± 0.29	4.34 ± 0.19	4.84 ± 0.19	5.35 ± 0.30	5.75 ± 0.22
A.J. (3 days) : A.N. (11 wk)	5.70 ± 0.33	5.22 ± 0.29	5.48 ± 0.28	5.80 ± 0.33	5.95 ± 0.35
H.C. (5 days) : E.R. (8 wk)	6.24 ± 0.37	7.04 ± 0.45	7.20 ± 0.50	7.35 ± 0.52	7.53 ± 0.55
H.C. (5 days) : J.N. (7 wk)	6.48 ± 0.40	5.88 ± 0.35	5.70 ± 0.27	5.92 ± 0.32	6.26 ± 0.18
H.C. (5 days) : H.C. (7 wk)	5.85 ± 0.34	5.24 ± 0.29	6.06 ± 0.39	6.86 ± 0.45	7.47 ± 0.47
Mean average	5.84 ± 0.29	5.52 ± 0.27	5.83 ± 0.29	6.15 ± 0.30	6.29 ± 0.31

[a] Aliquots of 0.10 ml of each serum, or normal saline were mixed, incubated at 37° for 30 min then 0.10 ml was added to the bathing medium containing the muscle contracting at a steady state in response to electrical stimulation. Each mixture was prepared on three separate days and assayed, the mean and the standard deviation are shown.

[b] Average of 10 separate experiments.

[c] The period given in parentheses indicates the period (days or wk) after thermal injury the blood was drawn.

When extracted with the two-phase butanol–glacial acetic acid system, and processed as described for the "toxic glycoprotein" (3, 15), the aqueous phase from extraction of serum of acute thermally injured patients contained also an inhibitor of ATP-mediated tension and depressed also the developed tension of rat heart papillary muscles contracting isometrically in response to electrical stimulation (15).

The serum from convalescent thermally injured patients caused from weak to no effect on the developed tension, and neutralized the effects of the serum from acute thermally injured patients. This observation supports the suggestion that the serum from thermal injury contained an inhibitor, and during convalescence, the acute thermally injured patient developed neutralizing anti-inhibitor(s).

Summary. Evidence is presented which demonstrates that serum from acute thermally injured subjects depresses the developed tension in rat papillary muscles contracting in response to electrical stimulation. The serum from normal volunteers, or from convalescent thermally injured patients slightly increased or had no effect on the developed tension. If combined with the serum from convalescent thermally injured subjects, the serum from the acute thermally injured subjects had no effect on the developed tension. These observations suggest the presence of a myocardial depressant in the serum of acute, and a neutralizing compound in the serum of convalescent thermally injured subjects.

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