Thermal Injury: Release of an Inhibitor of Adenosine 5′-Triphosphate Induced Muscular Contraction[1] (36315)

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Despite increasing recognition of the release of specific toxic substances by thermal injury, relatively little is known about the mechanism by which these substances exert their damaging effects on the host. A toxic glycoprotein prepared from *in vitro* scalded human skin inhibited the formation of adenosine 5′-triphosphate (ATP)-induced tension by glycerol-extracted muscle fibers (1, 2). The inhibition was independent of calcium or magnesium concentration, but dependent on the glycoprotein concentration (3). Rabbits injected with the toxic glycoprotein developed specific antibodies titers against the glycoprotein. Serum of convalescing burn patients, similar to rabbit antiserum, or its gamma globulin fraction, neutralized the glycoprotein physiological inhibitory activities (4). The sensitivity of the glycerol-extracted muscle fiber to adenosine 5′-triphosphate was inhibited by the "toxic glycoprotein," but was enhanced by an active protein present in normal human serum (5).

In this investigation, the effects of serum from "acute burn" and "convalescing burn" patients on the development of ATP-induced tension by glycerol-extracted fibers were studied. The inhibitory effect of the acute burn serum was determined at increasing concentrations, and at time intervals during the convalescing period. Neutralization of the inhibitory effect of the acute burn serum by "convalescing burn" serum was determined at intervals of time during the convalescing period. This paper demonstrates the presence of an inhibitor of ATP-induced tension in the serum of acute burn patients. The degree of inhibition was concentration dependent. The acute burn serum inhibitory effect was neutralized by convalescing burn serum.

Methods. Blood samples were obtained from Sumner 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 10 min. The serum was dialyzed against three 10-fold changes of 62 m*M* Tris–phosphate buffer, of pH 7.4, containing 2.5 m*M* $MgCl_2$, then was frozen at —20° in multiple aliquots for later analysis. Only the name of the patient was known at the time of analysis. Adenosine 5′-triphosphate disodium salt (99.9% pure) was obtained from Sigma Chemical Company and was used as 10^{-2} solution in 0.9% NaCl neutralized to pH 7.4. Only freshly prepared solutions were employed.

The toxic glycoprotein was prepapred from *in vitro* scalded human skin (6).

Glycerol-extracted muscle strands: Glycerination of rabbit psoas muscle bundles was carried out according to the method of Szent-Gyorgyi (7). The fibers were washed with 50% glycerol (—20°) daily for 7 days then stored at —20° until used. The glycerinated bundles were removed from the 50% glycerol, washed once with 62 m*M* Tris–phosphate buffer, of pH 7.4. The bundles were then divided into 0.1 mm by 60 mm strands and suspended from a grass model force transducer in 40 ml of 62 m*M* tris–phosphate buffer, of pH 7.4, containing 2.5 m*M* $MgCl_2$. The muscle strand was stretched to approximately 70 mg of positive tension; then either 0.9% NaCl, glycoprotein, or

[1] Presented: 1971 Meet. of the Fed. Amer. Soc. Exp. Biol. Fed. Proc., Fed. Amer. Soc. Exp. Biol. 30, 378 (1971).

TABLE I. Adenosine 5'-Triphosphate-Induced Tension in Glycerol-Extracted Fibers Incubated in Tris–Phosphate–$MgCl_2$ Buffer Containing 0.25 ml of Dialyzed Serum from Burn Patients.

Patient	Thermal injury		Assay date	ATP-generated tension[a] after ATP		
	Degree[b]	Date		1	2	3
G.B.	34	07.03.70	07.22.70	15	22	25
B.G.	35 (14)	06.30.70	07.22.70	12	16	18
M.G.	22 (1)	06.30.70	07.22.70	16	30	36
R.A.	51 (9)	06.30.70	07.22.70	15	16	16
J.S.	80 (50)	10.20.70	10.22.70	4	7	9
L.H.	33 (0)	10.19.70	10.22.70	25	43	45
I.D.	45 (8)	10.25.70	10.28.70	1	23	24
D.I.	45 ( )	10.25.70	10.28.70	3	24	25
L.S.	41 (15)	10.04.70	11.02.70	29	36	40
C.A.	38 (08)	10.06.70	11.13.70	22	59	59
J.E.	17 (3)	10.07.70	11.23.70	45	67	85
H.E.F.	33 (09)	01.18.71	01.18.71	12	40	60
Y.H.	33 (07)	11.07.70	11.10.70	10	42	28
S.L.R.	41 (15)	11.04.70	11.20.70	29	36	40
B.H.	47 (7)	11.07.70	11.10.70	1	24	45
A.R.	51 (9)	07.30.70	11.10.70	20	38	40
S.E.	60 ( )	12.10.70	12.10.70	3	10	20
G.A.	35 (1)	01.02.71	01.20.71	27	47	52
R.N.	35 (10)	01.11.71	01.20.71	12	25	28
R.U.	42 (14)	11.28.70	12.02.70	28	15	23
S.J.	46 (23)	12.05.70	12.11.70	7	10	20
J.C.	45 (40)	02.07.71	02.10.71	8	15	23
C.P.	50 ( )	02.01.71	02.10.71	9	15	32
P.M.	48 (24)	04.05.70	07.22.70	35	43	45
			11.02.70	37	42	72
			12.10.70	44	64	79
Saline			07.22.70	49	79	105
			10.22.70	48	77	102
			10.28.70	52	82	107
			11.02.70	59	97	122
			11.13.70	56	82	120
			11.10.70	67	112	121
			11.20.70	45	65	127
			12.10.70	51	74	96
			12.11.70	54	78	102
			12.02.70	58	94	126
			01.20.71	48	87	120
			02.10.71	57	75	92

[a] Tension was induced with three concentrations of aATP by adding 0.10 ml aliquots of 10^{-2} *M* ATP to the 40 ml of the Tris–phosphate–$MgCl_2$ buffer bathing the fiber.

[b] Degree of thermal injury as percentage of total body injured; ( ) = full thickness burn.

serum was added to the bathing medium, incubated at room temperature, *i.e.*, 26° for 5 min, or as indicated; then aliquots of ATP were added. All experiments were monitored at room temperature with a model 79 grass polygraph with a 7 P 1 preamplifier.

Results. In control experiments in which ATP was added in increments to glycerol-extracted fibers, the generation of the tension to the first addition of ATP was rapid. In 10

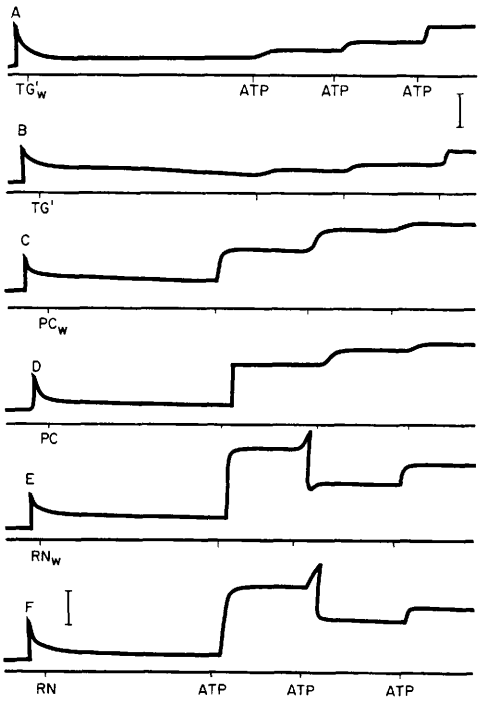

FIG. 1. Tracings of ATP-induced tension developed by unwashed and once washed glycerinated fiber incubated with the "toxic glycoprotein" [(A) and (B), respectively], unwashed and once washed fibers incubated with "acute burn" serum PC [(C) and (D), respectively] and unwashed and once washed fibers incubated with "convalescing burn" serum RN [(E) and (F), respectively]. The sharp break in (E) and (F) is a change in the sensitivity of the polygraph from 0.02 to 0.05 V/cm.

random experiments, the tension generation in response to the addition of the first aliquot of ATP ranged from 48.5 to 67.5 with an average of 55.1 pen deflection (each pen deflection was equivalent to 9 mg/cm^2). The variability of these results are characteristic of much of the work done with preparations of this kind (7, 12, 13). Subsequent addition of ATP to the glycerol-treated fibers, increased the developed tension to a maximum, most commonly attained after a final concentration of 7.6×10^{-4} M ATP. In the studies, three controls were tested for each experiment and the average generated tension was used in calculating the data. The greater variability of the maximum generated tension has led us to base the conclusion principally on results of the initial contraction generated by the first aliquot of ATP. The subsequent two additional ATP aliquots were employed to establish the physiological responsiveness of the fibers employed in each experiment. It is evident, however, that the maximum tension formed by a fiber depends on the final amount of the ATP added. All experiments described in Table I were run in triplicate. The average deviation was less than 10%.

The data summarized in Table I compare the inhibitory effect of each serum from 25 different burn patients with the magnitude of the thermal injury. When glycerol-treated fibers were incubated with 0.25 ml of freshly drawn dialyzed serum of normal adult volunteer (AAH), addition of ATP-generated tension of 42 ± 10; 62.5 ± 10, and 90 ± 15 pen deflections after the three aliquots of ATP, respectively.

When glycerinated fibers were tested under such experimental conditions to reveal possible presence of natural muscle proteins, the results obtained suggested that the glycerinated fibers employed contained troponin, tropomyosin, and sarcoplasmic reticulum fragments and free calcium in such concentrations not to influence the magnitude of the ATP-generated tension. For example, when washed or unwashed glycerol-extracted fibers were incubated with 0.25 mg of the "toxic glycoprotein," 95% inhibition of the generated ATP-induced tension was obtained with both fibers as shown in (A) and (B) of Fig. 1. If the washed or unwashed fibers were incubated with 0.25 ml of acute burn serum-PC (from 40% total body burn), 60% inhibition of the developed ATP-induced tension was suggested by (C) and (D) of Fig. 1. But, if the washed or unwashed fibers were incubated with 0.25 ml of convalescing burn serum-RN (week 8 after injury), 5% enhancement of the developed ATP-generated tension was suggested by (E) and (F) of Fig. 1.

When glycerol-extracted fibers were incubated with acute burn serum, the sensitivity of the fiber to ATP was inhibited to a degree dependent on the amount of the acute burn serum. Figure 2 shows the concentration de-

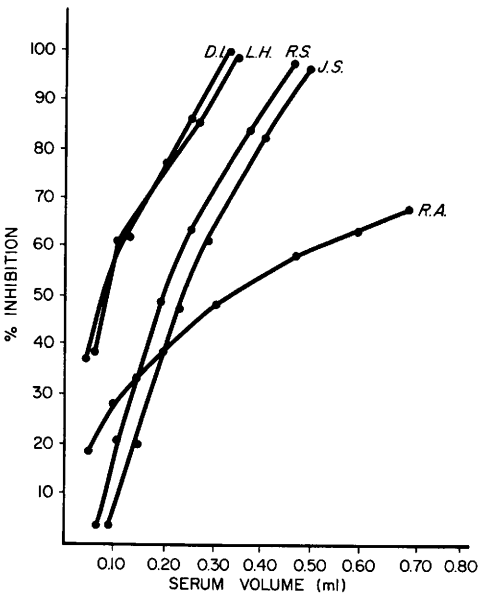

FIG. 2. Inhibition of ATP-induced tension with increasing concentrations of serum of acute burn patients. The decrease in tension as percentage of the tension formed by control fibers. The fibers were incubated with serum of burn patients DI, LH, RS, JS, and RA, with 45, 33, 41, 81, and 40% total body burns.

pendence curves for typical sera from 5 out of 12 fatally thermally injured patients 45 to 80% total body burn. The patients expired within the first week the blood samples were drawn. In all 12 cases, the data indicated high magnitude of inhibition of ATP-generated tension.

The data depicted in Fig. 3 suggests that the degree of inhibition of ATP-generated tension, by an acute burn serum from patient with 50% total body burn, depended on the incubation period with the fiber. When the acute burn serum and ATP were added simultaneously, inhibition of generated tension was less than 10%. As the incubation period was extended, the percentage of inhibition increased to a maximum at 4 min of incubation.

When glycerol-extracted fibers were incubated first with an acute burn serum, then simultaneously convalescing burn serum was added, and incubation was continued for a total of 5 min, addition of the standard amount of ATP caused 15 to 20% enhancing effect. Figure 4 shows the generated tension developed with the same amount of ATP by glycerol-extracted fibers incubated with acute burn BH or YH, and convalescing burn PM serum. The convalescing burn PM serum was added at varying times during the incubation period. This experiment suggested neutralization of the inhibitory effect of the acute burn BH or YH sera with the convalescing burn PM serum. If the fibers were incubated first with the acute burn serum BH, then normal AAH serum was added at varying times of the incubation period, the developed tension indicated a degree of inhibition dependent on the concentration of the acute burn serum. Normal serum did not influence the inhibitory effects of the acute burn serum or of the toxic glycoprotein.

The effect of acute burn and convalescing burn serum on ATP-contracted fibers is shown by (c) and (a) of Fig. 5. Trace (a)

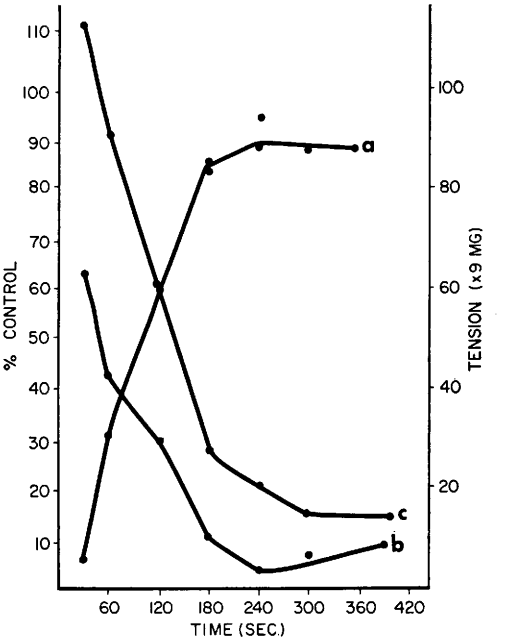

FIG. 3. Inhibition of development of ATP-induced tension with acute burn serum from a 50% total body burned patient: (A) the decrease in tension as percentage of the tension developed by control fiber at 2.5×10^{-5} *M* ATP; (B) and (C) the tension in pen deflections (multiplied by 9 will give mg/cm^2) developed at 2.5×10^{-5} *M* and 5.0×10^{-5} *M* ATP.

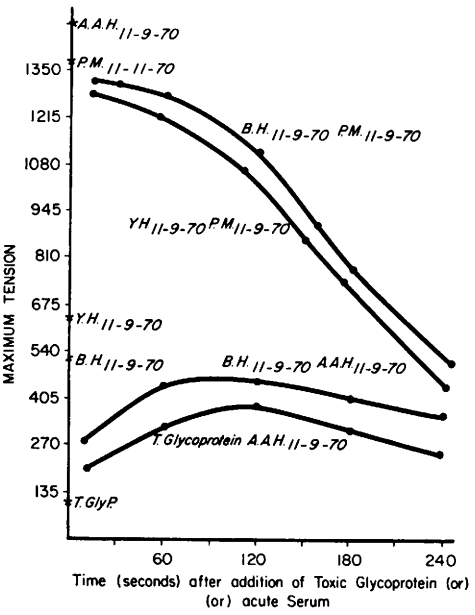

FIG. 4. Neutralization of the acute burn serum inhibitory activity with convalescing burn serum. The fibers were incubated first with 0.25 ml of the acute burn (BS or YH) serum for the time indicated, then 0.25 ml of the convalescing burn (PM), or normal AAH serum was added. Total incubation time was 300 sec. The maximum tension developed was at 2.5×10^{-5} *M* ATP (mg/cm^2).

shows the effect of 0.10 ml of the serum PW convalescing burn on an ATP-contracted fiber. The sharp break in the tracing is a change in polygraph sensitivity from 0.02 to 0.05 mV/cm. Addition of convalescing burn serum-PW caused slight relaxation of contracted fiber. The fiber was relaxed by a purified enzyme concentrate (EC). Tracing (b) demonstrates a typical response of ATP-contracted fiber to 0.05 mg of the toxic glycoprotein. A sharp loss of tension resulted immediately after addition of the glycoprotein to the bathing medium. The enzyme concentrate (EC) caused similar relaxing effect. When the ATP-contracted fiber was incubated with acute burn serum-JC, trace (c) suggests progressive relaxation of the fiber. When the ATP-contracted fiber was incubated with convalescing burn serum PW, addition of the toxic glycoprotein had no relaxing effect, but the purified enzyme concentrate (EC) produced immediate relaxation of the fiber shown in Fig. 5d. When the ATP-contracted fiber was incubated with the "acute burn" serum JC, addition of the toxic glycoprotein caused immediate relaxation of the fiber as suggested in (e). In these experiments, the purified enzyme con-

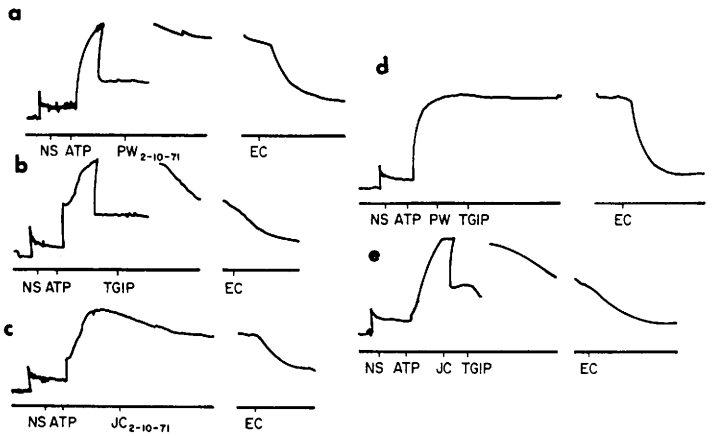

FIG. 5. Neutralization of the toxic glycoprotein or of the acute burn serum relaxing effect with convalescing burn serum: (a, b, and c) the relaxing effect of 0.10 ml convalescing serum-PW, 0.05 mg of toxic glycoprotein-TGLP, or 0.10 ml of the acute burn-JC serum on ATP, tensed fibers, respectively. (d and e) the neutralization of the toxic glycoprotein with convalescing burn serum, and the combined effects of the toxic glycoprotein with the acute burn serum, respectively. The sharp break in the tracings show changes in sensitivity of polygraph from 0.02 to 0.05 then back to 0.02 mV/cm^2.

centrate (EC) was added to determine the physiological state of the fiber after the action of the serum or of the glycoprotein.

Discussion. The work of Rosenthal *et al.* (8, 9) and many other investigators (10, 11) suggested the presence of a specific burn toxin as well as an antitoxin. This study, an approach to investigate the physiological effects of an inhibitor of ATP-induced tension, showed that the serum of acute burn patients contained an inhibitor of ATP-induced tension and caused the relaxation of the ATP-contracted muscle strand. The magnitude of inhibition depended on the serum employed, *i.e.*, degree of burn, the incubation time with the muscle strand, and the time of injury. The serum of convalescing burn patients produced weaker to no inhibitory effects on the generation of ATP-induced contraction. The data suggested that the variant was the period of convalescence. When added before or after, the convalescing burn serum neutralized the inhibitory effects of the acute burn serum or of the toxic glycoprotein. Employing the glycerol-extracted muscle strand as a model for the site of action of the toxic glycoprotein, data have been obtained to substantiate the release of such substances into the serum of an acute burn serum patient.

The principal author (A.A.H.) gratefully acknowledges with thanks Dr. P. Hawley of the Department of Physiology for the use of his laboratories, and Dr. John Bazwick and his staff for his continuous support of these studies by contributing samples of the blood of the patients of the Burn Ward.

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Received May 24, 1971. P.S.E.B.M., 1972, Vol. 139.