mortality are seen within 48 hours of exposure. The first period, 3-9 hours post-exposure, is characterized by an approximately linear dose-mortality response above 750 r. In the second phase (17-30 hours) approximately 40% of the animals irradiated with 750 r died, no increase being observed with higher radiation doses.

1. Bacq, Z. M., Alexander, P., *Fundamentals of Radiobiology*, 2nd ed., Pergamon Press, N. Y., 1961.
2. Tyler, S. A., Stearner, S. P., *Int. J. Rad. Biol.*, 1962, v4, 495.
3. Jacquez, J. A., Karnofsky, D. A., *Am. J. Roentgenol. and Rad. Therapy*, 1950, v64, 289.
4. Stearner, S. P., Christian, E. J., Brues, A. M., *Rad. Res.*, 1954, v1, 270.
5. Stearner, S. P., Sanderson, M. H., Christian, E. J., Brues, A. M., *Am. J. Physiol.*, 1958, v192, 620.
6. Patt, H. M., Tyree, E. G., Straube, R. L., Smith, D. E., *Science*, 1949, v110, 213.
7. Thomson J. F., *Radiation Protection in Mammals*, Reinhold Publ. Co., N. Y., 1962.
8. Beaumariage, M. L., *Compt. Rend. Soc. Biol.*, 1957, v151, 1788.
9. Stearner, S. P., Christian, E. J., Brues, A. M., *Am. J. Physiol.*, 1954, v176, 455.

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Action of Ouabain on Isometric Twitch Tension in Striated Muscle.* (28537)

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While the cardinal effects of the cardiac glycosides are manifested on heart muscle in congestive heart failure, there is evidence that these agents also affect extracardiac organs(1). The purpose of this study was to determine the action of g-strophanthidin (ouabain) on the isometric twitch tension of non-cardiac striated muscle. The rat diaphragm was chosen as a convenient preparation since it lends itself well to *in vitro* studies of drugs affecting muscle tension. This may be determined either by direct muscle stimulation or by indirect muscle stimulation *via* the phrenic nerve.

It was proposed to study the inotropic effects of ouabain on the isometric twitch tension of the indirectly stimulated rat phrenic nerve-diaphragm preparation and to correlate the results with the electrolyte (Na, K, Ca) changes in the diaphragm.

Methods. Adult male albino rats weighing 150 to 200 g were used. Isolated phrenic nerve-diaphragm preparations, according to the method of Bülbring(2), were immersed in 50 ml of oxygenated Tyrode solution at 27°C, containing 0.2% glucose. Tissue electrolyte determinations were made on both treated and control segments of diaphragmatic muscle.

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The nerve-diaphragm segment was firmly fixed by 2 metal hooks to a muscle holder at the costal margin. The phrenic nerve was placed in a chamber containing 2 platinum electrodes. A constant tension of 2 g was allowed to act on the diaphragm. For isometric twitch tension experiments, the tendinous end of the diaphragm segment was immobilized and attached to a Grass force-displacement transducer, model FT 03. The isometric twitch tension was recorded on a polygraph with a direct current drive amplifier. The median values of the developed tension in 30 consecutive isometric twitches were selected to determine dose-response relationships. For isotonic contractions the tendinous end of the muscle was attached to a light writing lever and the contractions recorded on smoked paper. The diaphragm segment was indirectly stimulated *via* the phrenic nerve every 10 seconds for a period of 5 minutes with single supra-maximal (10 Volt) direct current square wave stimuli of 0.25 millisecond duration.

A Beckman DU spectrophotometer with flame attachment was used for the electrolyte analyses. The tissue cations were expressed as mg % of dried weight. The paired *t* test was employed to determine the signifi-

TABLE I. Effect of Increasing Doses of Ouabain on Tension Developed During Isometric Contractions of Indirectly Stimulated Rat Diaphragm.

	Ouabain			
Control	(0.5 μg/ml)	(1 μg/ml)	(2 μg/ml)	(4 μg/ml)
4.88 ± .156*	5.32 ± .190	5.72 ± .260	6.12 ± .260	6.68 ± .267

*Mean ± S.E. of isometric twitch tension in grams for 10 experiments.

cance of alteration in electrolyte concentration after stimulation and/or drug treatment.

Experiments were performed on the resting diaphragm exposed to ouabain, the indirectly stimulated diaphragm in the absence of ouabain, and the indirectly stimulated diaphragm in the presence of ouabain.

In the first series a period of 15 minutes was allowed for equilibration, after which time the Tyrode solution was replaced. After a 15-minute control period, ouabain was added every 15 minutes in such increments as to make a final concentration of (0.5 μg/ml), (1 μg/ml), (2 μg/ml) and (4 μg/ml) respectively. Immediately following 15 minutes of exposure to (4 μg/ml) of ouabain both diaphragms, treated and control, were removed from their respective muscle baths and blotted with filter paper. The weighed samples were placed in platinum crucibles for ashing. The residual ash of the samples was analyzed for electrolyte content (Na, K, Ca) to determine the effects of exposure to ouabain on the electrolyte content of the resting muscle.

In the second series following a period of equilibration the diaphragm was indirectly stimulated every 15 minutes for a period of 5 minutes. During each 5-minute period of stimulation 30 twitches were recorded. The duration of each experiment was 75 minutes. The stimulated and control segments of the diaphragm were analyzed for electrolyte content (Na, K, Ca) to determine the effects of stimulation on the electrolyte content of the muscle.

In the third series the diaphragm was indirectly stimulated for periods of 5 minutes at each dose level of ouabain (see above) during which time 30 twitches were recorded. The ouabain-treated and control segments of the diaphragm were analyzed for electrolyte content (Na, K, Ca) to determine the effects of ouabain plus stimulation on the electrolyte content of the muscle.

Results. Ouabain in increasing increments significantly potentiated the median isometric twitch tension of the rat diaphragm (Table I). The potentiation of twitch tension was dose-dependent. With a maximal concentration of ouabain (4 μg/ml) there was a 38% increase in isometric twitch tension as compared to the initial control. In no case did fatigue or contracture of the muscle occur during the experiments.

Table II summarizes the changes in electrolytes in the resting rat diaphragm as a result of exposure to ouabain (series 1), following stimulation (series 2), and following ouabain plus stimulation (series 3). Administration of ouabain in increasing increments until a final concentration of 4 μg/ml was reached caused a significant increase in the sodium content of the muscle, a decrease in potassium and no significant change in calcium. In the second series, stimulation alone produced a significant increase in Na and Ca and a significant decrease in K. In the third series, stimulation in the presence of ouabain, there was a significant increase in Na and Ca and a significant decrease in K.

Discussion. Stimulation of the rat diaphragm resulted in uniform isotonic contractions agreeing with the observations of several investigators(2,3,4) who demonstrated that both isotonic and isometric contractions of the isolated rat diaphragm remained constant for several hours if the rate of stimulation was approximately 6 shocks/min.

The isometric twitch tension was augmented by increasing dose levels of ouabain. The optimal muscle length for maximal contraction of the rat diaphragm was not determined. Since the muscle length was kept uniform, augmentation of isometric tension due to variation in initial muscle length as

TABLE II. Effect of Ouabain, Indirect Stimulation and Indirect Stimulation Plus Ouabain on Sodium, Potassium and Calcium Content of Diaphragmatic Muscle.

	Experimental			Control			% change	
Electrolyte	n	M*	S.E.	n	M*	S.E.	from control	P
Series 1		Ouabain†						
Na	10	536	17.6	10	509	20.5	+ 5.72	<.001
K	10	887	22.8	10	915	21.8	— 3.04	<.01
Ca	10	60.8	3.73	10	58.0	2.82	+ 4.69	>.25
Series 2		Stimulation						
Na	10	545	24.0	10	498	26.1	+10.1	<.001
K	10	878	18.1	10	939	21.7	— 6.36	<.001
Ca	10	64.8	2.53	10	61.0	2.88	+ 6.72	<.01
Series 3		Stimulation + Ouabain†						
Na	10	555	17.7	10	492	21.6	+12.8	<.001
K	10	856	27.3	10	923	34.5	— 7.28	<.001
Ca	10	67.7	3.48	10	62.0	2.82	+ 9.11	<.001

* Mean concentration of cations in mg % of dried weight.
† Final concentration (4 μg/ml).

observed by Rosenblueth *et al.*(5,6) was avoided.

The positive inotropic action of cardiac glycosides on striated muscle has been illustrated infrequently in the literature. Del Pozo and Pardo(7) found a positive inotropic effect of k-Strophanthoside on ischemic cat gastrocnemius muscle. Cattell(1) noted an increase isometric twitch tension of frog sartorius muscle after ouabain which corresponded to the inotropic effects of ouabain on the myocardium.

Greeff and Westermann(8) demonstrated that toxic doses of ouabain (15 μg/ml) greatly impaired neuromuscular transmission in the rat diaphragm preparation as determined by indirect stimulation of the muscle. Also, this dose rendered the muscle incapable of responding to direct electrical stimulation. To demonstrate ouabain-induced paralysis of the indirectly stimulated diaphragm it was necessary to elevate the potassium content of the Tyrode solution from 10 mg% (2.5 mM) to 30 mg% (7.5 mM). After a brief improvement of muscle contraction they noted a distinct decline in direct and indirect excitability. The inhibition of direct excitability was also demonstrated with potassium alone (50 mg%). The inhibitory effects of ouabain and potassium were antagonized by calcium chloride, epinephrine and norepinephrine. These investigators found a correlation between maximal ouabain dose, maximal potassium loss, maximal paralysis and maximal muscle temperature depression. The diaphragm differs from cardiac muscle in its response to ouabain at high levels of potassium. Potassium potentiated the toxic effects of ouabain on the diaphragm(8); however, in mammalian ventricular muscle a wide range of potassium levels (3.5 to 8.5 mM) did not alter the positive inotropic action of ouabain (9). The observation(8) that calcium antagonized the effect of ouabain on the rat diaphragm is in contrast to its potentiating effect on ouabain in cardiac muscle(10).

In our experiments ouabain-induced paralysis of the rat diaphragm did not occur since the Tyrode solution contained 2.5 mM of potassium and the concentration of ouabain did not exceed 4 μg/ml.

Calcium content of the frog sartorius muscle has been shown to increase following direct stimulation(11). In our results the calcium content of the stimulated diaphragm, in the presence or absence of ouabain, was significantly increased.

A loss of potassium was noted in the stimulated diaphragm either in the presence or absence of ouabain and also in the resting diaphragm exposed to ouabain. A loss of potassium has been noted in the cat gastrocnemius muscle following stimulation(12) and in the resting rat diaphragm exposed to 15 μg/ml of ouabain(8).

A net loss of tissue potassium was associated with a net gain in sodium in all 3 experimental series. Expressed on the basis of

mEq/100 g of dried tissue, the gain in sodium was proportionately greater than the loss of potassium. These results are in essential agreement with those of Fenn *et al.*(12) who demonstrated a net loss of potassium and a net gain in sodium in the stimulated cat gastrocnemius muscle(12).

Various physiological factors may be considered involved in skeletal muscle activity; (a) the propagated action potential causing (b) active cation transport, (c) with the activation energy or "alpha process" causing (d) an alteration in the myosin contractile elements which results in (e) tension development and/or muscle shortening. Drug-induced potentiation of muscle contraction may occur at either of these rate limiting stages. The experimental design of this study was not such as to permit a definitive explanation of the mode of action of ouabain in potentiating the isometric twitch tension of the rat phrenic nerve-diaphragm preparation. While it was demonstrated that significant changes in sodium and potassium occurred in all 3 experimental series the greatest shifts in Na, K and Ca occurred in the stimulated muscle in the presence of the cardiac glycoside. Some investigators(11) reason that changes in calcium may be more significant than changes in sodium and potassium as regards striated muscle activity. These authors cited indirect evidence suggesting that the entry of calcium into the muscle fiber is closely associated with the development of muscle action potentials and hence muscle contraction. A study designed to relate shifts in calcium to the isometric twitch tension of striated muscle, incident to exposure to digitalis glycosides, may shed further light on the mode of action of such drugs in increasing skeletal muscle activity.

Summary. Ouabain significantly increased the isometric twitch tension of the indirectly stimulated rat diaphragm. The diaphragm was analyzed for sodium, potassium and calcium content following exposure to ouabain, exposure to ouabain plus indirect stimulation of the muscle, and the effect of indirect stimulation alone. 1. In the resting diaphragm increasing increments of ouabain (0.5, 1, 2 and 4 μg/ml) resulted in a significant gain in muscle Na, a significant loss of K but no change in Ca as compared to the resting, non-ouabain treated muscle. 2. In the stimulated diaphragm the same increments of ouabain resulted in a significant increase in Na and Ca content of the muscle and a significant loss in K as compared to the resting, non-ouabain treated muscle. In the control stimulated diaphragm there was also a significant gain in Na and Ca content of the muscle and a significant loss in K as compared to the resting, non-ouabain treated muscle. The greatest shifts in Na, K and Ca occurred in the stimulated muscle in the presence of the cardiac glycoside.

1. Cattell, M., *J. Pharm. & Exp. Therap.*, 1938, v62, 459.

2. Bülbring, E., *Brit. J. Pharm. & Chemother.*, 1946, v1, 38.

3. Creese, R., Hashish, S. E. E., Scholes, N. W., *J. Physiol.*, 1958, v143, 307.

4. West, G. B., *Quart. J. Pharm.*, 1947, v20, 518.

5. Rosenblueth, A., Alanis, J., Rubio, R., *Arch. Int. de Physiol. et de Biochim.*, 1958, v66, 330.

6. ———, *ibid.*, 1958, v66, 354.

7. del Pozo, E. C., Pardo, E. G., *J. Pharm. & Exp. Therap.*, 1949, v97, 144.

8. Greeff, K., Westermann, E., *Arch. f. Exp. Path. u Pharmakol.*, 1955, v226, 103.

9. Garb, S., Venturi, V., *J. Pharm. and Exp. Therap.*, 1954, v112, 94.

10. Salter, W. T., Sciarini, L. J., Gemmel, J., *ibid.*, 1949, v96, 372.

11. Bianchi, C. P., Shanes, A. M., *J. Gen. Physiol.*, 1959, v42, 803.

12. Fenn, W. C., Cobb, D. M., Manery, J. F., Bloor, W. R., *Am. J. Physiol.*, 1938, v121, 595.

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