A COMPARATIVE STUDY ON THE ELECTRICAL AND MECHANICAL PROPERTIES OF HUMAN AND GUINEA PIG ATRIAL MUSCLES IN VITRO

SUKENOBU ITO

IN 1957, *Woodbury et al.*¹ succeeded in recording transmembrane action potential from human ventricular muscle exposed during cardiac surgery, and *Trautwein et al.*² recorded the action potential on the human atrial and ventricular myocardium isolated from hearts of patients undergoing corrective open-heart surgery. *Sleator* and *de Gubareff*³ observed unique contraction patterns and action potential configurations in a series of studies on human atrial muscle at the temperature below physiological range (24–27°C). *Prasad*⁴ has demonstrated the electrophysiological behavior of isolated human papillary muscle in association with varying concentrations of extracellular potassium and ouabain. Thus, many electrophysiological studies on the human cardiac muscle have been published,^{1–14} and these studies have contributed a great deal to the understanding of the physiology of the human heart as well as clinical cardiology.

However, the electrophysiological properties of human myocardium would be understood better by a study in comparison with other mammals, since the electrophysiological characteristics of cardiac tissue vary from one species to another. The present paper is concerned with a comparable study of the transmembrane action potential and the contractile characteristics of isolated atrial tissues of man and guinea pig, with special reference to the responses to different

stimulus rates, extracellular ion concentrations, and dose-response relationship of ouabain.

METHOD AND PROCEDURE

One hundred and thirty-two strips of human atrial muscle were obtained from the following patients of heart diseases who received corrective heart surgery in the First Department of Surgery of the Kyushu University: 43 ventricular septal defect, 43 atrial septal defect, 15 tetralogy of Fallot, 3 pulmonary stenosis, 4 patent ductus arteriosus, 3 endocardial cushion defect, 1 total anomalous pulmonary venous drainage, 1 double outlet of right ventricle, 1 aortic septal defect, 17 mitral valve diseases, and 1 aortic insufficiency. The age distribution of these patients ranged from 7 months to 32 years. Most of the specimens were excised from right atria during the procedure of open-heart surgery with the heart-lung by-pass machine. In addition, some segments (7.6%) were obtained from left atria of the patients with mitral stenosis and patent ductus arteriosus.

Immediately after excision, the tissues were immersed in oxygenated Tyrode solution (temperature: 30°C) and dissected into muscle strips which were prepared in such a way that the most prominent fiber bundles run parallel to their length. They were mounted in an acrylic perfusion vessel (containing 30 ml solution) and secured to the rubber bottom of the chamber by stainless steel pins (Fig. 1). The strips were perfused with Tyrode solution at a flow rate of 10 ml/min. The solution had the following composition (in mM): NaCl 136.8, KCl 2.68, CaCl₂ 1.82, NaHCO₃ 11.9, MgCl₂ 0.49,

Key Words:

Human atrial muscle
Transmembrane action potential
Force-frequency curve
Cardiac glycoside
Hypokalemia

(Received on March 22, 1974; Accepted for publication: August 28, 1974)
1st Department of Medicine, Faculty of Medicine, Kyushu University
(Director: Prof. Toshiyuki Yanase)

Fig.1. A diagrammatic representation of experimental arrangement.

Fig.2. Typical transmembrane action potential of isolated human (A, B) and guinea pig (C) atrial muscles. Horizontal line indicates zero potential.

NaH_2PO_4 3.3, and glucose 5.56. It was oxygenated continuously with 95% O_2 -5% CO_2 of pH, 7.6, and kept at 30°C by a thermostat.

A preliminary study revealed that contractile force rapidly declined and failed spontaneously at a temperature higher than 30°C . A thread with small suture was tied to one end of the strips for recording of the isometric contraction. The muscle was electrically stimulated by supra-threshold field stimuli¹⁵ of 5 msec duration at a rate of 60/min.

The transmembrane action potential was recorded with a Ling-Gerald typed capillary glass microelectrode filled with 3 M KCl, which had tip smaller than 0.5μ in diameter and resistances

of 20 – 30 megohms. The force of contraction was recorded with a strain-gauge transducer. The transmembrane action potential and force of contraction were simultaneously recorded on a VC-6 NIHON KODEN oscilloscope and ink-writing oscillograph, and also photographed on 35 mm film. The experimental protocol was as follows: the muscles were equilibrated in normal Tyrode solution with a stimulus rate of 60 per minute for at least one hour prior to recording of the electrical and mechanical activities. Then, the effects of stimulus frequency, varying concentrations of extracellular potassium and calcium, and dose-response relationship of ouabain were observed.

Fig.3. The force-frequency relationship of isolated human (A) and guinea pig (B) atrial muscles. The solid line was obtained from the good preparations and the dotted line from "hypodynamic" ones.

Under the same conditions, the same procedures were done for the left atrium obtained from guinea pig. That is, guinea pigs weighing 250–350 gm were killed by stunning, and the left atrium was rapidly excised. The preparation was fixed at one end on a rubber block in the bath perfused with the oxygenated Tyrode solution at a temperature 30°C, and the other end was connected at a strain gauge to measure the isometric contraction. The guinea pig transmembrane action potential and force of contraction were recorded just as a same manner as in the human studies.

RESULTS

For 39 of 132 human atrial muscle strips (29.5%), simultaneous recordings of transmembrane action potential and the contractile force were successful. For the most of the unsuccessful

Fig.4. Effect of change of extracellular calcium concentration on the force-frequency relationship curves of isolated "hypodynamic" human (A) and guinea pig (B) atrial muscles. When extracellular calcium concentration was increased, marked increase of contractile force was observed, especially at the higher frequency of stimulation, and the monophasic or biphasic curves usually seen at "hypodynamic" preparations changed to typical triphasic pattern.

cases, the tissues were from patients of younger age yielding muscle too small for study. No special correlation was found between the nature of diseases and the behavior of isolated atrial muscle under the present experimental conditions.

Configuration of transmembrane action potential

The isolated human atrial muscles showed two different types of transmembrane action potentials (Fig. 2 – A, B): Type A and B. As can be seen in Fig. 2, type A is characterized by a typical contour similar to that seen in atrial fibers of guinea pig (Fig. 2 – C), having no

Fig. 5. Effect of change of extracellular potassium concentration on the force-frequency relationship curves of isolated human (A) and guinea pig (B) atrial muscles. Increased force of contraction was recorded at the lower concentration of potassium, and the typical triphasic curves changed to monophasic pattern.

plateau and rapid repolarization; in contrast, type B is characterized by a prominent spike and plateau at a negative membrane potential. The latter type is commonly found in rather damaged

preparations. In Table I and II are shown the characteristics of transmembrane action potential of these two types in the human atrium. As for 60 percent repolarized duration, type A is significantly greater than type B ($p < 0.01$), but no other significant difference is seen.

Effects of stimulus rates and extracellular ionic concentrations

Fig. 3 shows the effects of stimulus rate on the contractile force of human (A) and guinea pig (B) atrial muscles. When the stimulus rates were increased from 10/min. to 200/min., most of the preparations showed a triphasic pattern characteristic of other mammalian atria.^{16,17} However, some preparation showed a monophasic pattern; namely the contractile force progressively decreased as the stimulus rates were progressively increased. These preparations were considered as hypodynamic ones as suggested by Levy.⁷

When the concentration of extracellular calcium was increased in the hypodynamic preparations, the force-frequency curves which was originally monophasic or biphasic changed to the typical triphasic pattern, and the optimum stimulus rate for contractility shifted to the higher stimulus rate for both the human and guinea pig atrium (Fig. 4 - A, B).

When the concentration of extracellular potassium was reduced, the contractile force became larger, especially at the low stimulus rates showing a monophasic pattern in response to increased frequency of stimulation (Fig. 5 - A, B).

Fig. 6 shows effects of various concentrations of extracellular calcium, potassium and sodium on the transmembrane action potential of human atria at the rate of 60 per minute stimulus frequency. Under low calcium concentration the plateau phase was prolonged, whereas under high

TABLE I AMPLITUDE OF TRANSMEMBRANE POTENTIALS IN VITRO OF HUMAN ATRIAL MUSCLE

		No. of cases	Max. (mv)	Min. (mv)	Mean (mv)	S.D.	t
Action Potential	A	16	127.0	80.0	101.2	13.95	1.491
	B	10	120.0	80.0	93.5	10.64	
Resting Potential	A	16	90.0	60.0	76.1	10.36	0.641
	B	10	85.0	60.0	73.5	9.53	
Overshoot	A	16	37.0	16.0	25.1	5.37	1.901
	B	10	35.0	12.0	20.0	8.37	

TABLE II DURATION OF TRANSMEMBRANE POTENTIALS IN VITRO OF HUMAN ATRIAL MUSCLE

		No. of cases	Max. (msec)	Min. (msec)	Mean (msec)	S.D.	t
30% Duration	A	16	48.0	13.8	34.1	10.24	1.673
	B	10	54.0	14.3	26.9	11.34	
60% Duration	A	16	266.6	113.4	185.7	46.38	8.009
	B	10	96.0	28.6	57.0	25.49	
90% Duration	A	16	576.0	307.1	424.3	71.12	1.704
	B	10	539.0	275.0	370.2	90.07

Fig. 6. Effect of change of extracellular calcium, potassium, and sodium concentrations on the transmembrane action potential of isolated human atrial muscles.

(1) Ca-free solution, (2) control solution, (3) Ca-5 X solution, (4) K-free solution, (5) control solution, (6) K-5 X solution, (7) Na-free solution, (8) control solution. No action potential was recorded at the higher concentration (2X) of sodium.

calcium phase 1 was accelerated, and phase 3 prolonged (Fig. 6 - 1, 2 and 3). The low concentration of potassium resulted in an increase in resting potential, and high concentration of potassium in a decrease in resting potential and reduction in amplitude (Fig. 6 - 4,

5 and 6). When the concentration of extracellular sodium was reduced by substitution of sucrose for NaCl, the amplitude decreased remarkably (Fig. 6 - 7, 8). At a higher concentration of extracellular sodium (2X) on the other hand, no action potential was recorded probably because

Fig. 7. Effects of increasing concentrations of ouabain on the transmembrane action potential and force of contraction of isolated human atrial muscles. (A) control in normal Tyrode solution, (B) 10^{-8} M ouabain, (C) 10^{-7} M ouabain, (D) 10^{-6} M ouabain, (E) after 15 minutes washing. An increase in concentrations of ouabain was carried out cumulatively every 15 minutes.

of higher osmolality.

Effects of ouabain on transmembrane action potential and force of contraction

In the present experiment concentration of ouabain was increased every 15 minutes, and cumulative curve of concentration-response relationship was observed over the range of 10^{-8} to 10^{-6} M concentrations of ouabain (Fig. 7). As a consequence the duration and amplitude of action potential of human atrium showed no significant change, or slightly increased at low concentration of ouabain, and decreased at high concentration (11.1%, 23.2%, respectively—Fig. 8—A, B). Similar changes were observed also in the guinea pig (Fig. 8—C, D).

On the other hand, a progressive increase was observed in the force of contraction without any significant change in the time to peak tension (Fig. 9).

Ouabain effect in potassium-free solution

When the concentration of ouabain was

increased in the same way as mentioned above after being exposed to KCl-free Tyrode solution for 15 minutes, change in contractile force of the human and guinea pig atrial muscles for various concentrations was somewhat different: the contractile force became maximum at lower concentration of ouabain as compared with that in control extracellular potassium concentration, and further increases in ouabain concentration induced ectopic impulse formation (Fig. 10).

DISCUSSION

From an electron-microscopic observation, Stein *et al.*¹⁸ reported that no significant correlation was found between the nature of the cardiac disease and the behavior of the isolated human atrial muscle. Also in the present study, no correlation was observed between the isolated human atrial tissues characteristic and the cardiac diseases. Sleator *et al.*³ failed to prepare specimens available in their experiment only for 20 of

Fig.8. Effect of ouabain on transmembrane action potential (TAP) characteristics of isolated human (A, B) and guinea pig (C, D) atrial muscle.

(A) % change of control of TAP amplitude of human atrial muscle. (B) % change of control of TAP duration (90%) of human atrial muscle. (C) % change of control of TAP amplitude of guinea pig atrial muscle. (D) % change of control of TAP duration (90%) of guinea pig atrial muscle. Each plotted from the average of 3 experiments.

220 human atrial muscles. In the present study 39 of 132 cases (29.5%) was successfully studied by simultaneous recording of transmembrane action potential and force of contraction. This discrepancy may be attributable to difference in the experimental procedures. Most of the unsuccessful cases were ascribable to the small amount of tissue from patients of younger age groups which had little pectinate muscles.

The configurations of the transmembrane action potentials obtained in the present study resembled those reported by other investigators^{2,3,19,20} but the duration of the action potential was rather longer than those reported by others. The latter difference may be attributable to difference in temperature in the experi-

mental condition and frequency of stimulation.²¹

Since *Bowditch* (1871), attention has been focussed in the effects of the interval between contractions on the strength of myocardial contraction.^{16,17,22-24} In general it is said that the force-frequency relationship in the atrial muscle of mammalian species is very similar, showing triphasic or "S shaped" pattern²⁴⁻²⁶ with the single exception being seen in rat atrium. In this connection *Hollander*²⁷ suggested that this might be due to differences in calcium utilization which were modified by stimulation frequency. *Kruta*²⁶ also pointed out that contractility optimum and potentiation were small and rather unstable in the rat myocardium as compared with other mammals. However, this

Fig. 9. Concentration-response curves of ouabain on isolated human atrial muscle. Note a progressive increase in the developed tension without any significant change in the time to peak tension with cumulative increase of concentration of ouabain.

force-frequency relationship is thought to vary depending upon temperature, range of stimulus frequency, and perfusing media^{1,7,24,27}. In the present study most of the human atrial muscles showed a triphasic force-frequency curve similar to those of the guinea pig and other mammalian atria. Those showing only monophasic or biphasic curve in hypodynamic preparations, reverted to the same triphasic pattern when the extracellular calcium concentration was raised, and maximum contractility was recorded at high frequency of stimulation. Winegrad and Shan-^{es}²⁸ and Grossman²⁹ demonstrated that in association with increases in frequency there was an increase in ⁴⁵Ca exchange or ⁴⁵Ca uptake as well as an increase in contractile amplitude, though the relationship between increases in exchange and contractility was not a direct one. Koch-Weser¹⁷ stated that this fundamental shape of force-frequency curve was due to the rapid phase of Negative Inotropic Effect of Activation (NIEA) decay and the longer duration of the Positive Inotropic Effect of Activation (PIEA) effect. Levy⁷ stated that the increased force of contraction seen with increase in frequency of beating was partly mediated through the release of endogenous norepinephrine. The force-frequency curve may thus be the result of many

Fig. 10. Effect of change of extracellular potassium concentration on dose-response curves of ouabain of human (A) and guinea pig (B) atrial muscles. The values are expressed as percent change from control. In the low concentration of potassium (1/2 of control), the contractile force became maximum at lower concentration of ouabain than in the normal potassium concentration, and further increase of concentration induced the formation of ectopic impulse. Parallel line (—||—) shows the ectopic impulse formation.

complicated factors.

Since Schatzman demonstrated the inhibition of Na and K active transport across red blood cells membranes by cardiac glycosides³⁰, the inhibition of the Na-pump by digitalis has been experimentally ascertained by many investiga-

tors.³¹⁻³⁹ Glynn⁴⁰ suggested that the positive inotropic effect of digitalis was most likely due to the inhibition of the Na-pump which enhanced the concentration of intracellular Na, and this, in turn, displaced Ca from the carriers allowing "free Ca" to exert its action on the contractile elements. Thus, the effect of digitalis on transmembrane action potential and force of contraction are thought to reflect the effects on membrane permeability and fluxes of Na, K, and Ca ions.

On the other hand, Wilbrandt and Weins⁴¹ reported that moderate or higher doses of cardiac glycoside inhibit Na transport across the frog skin, but very low doses sometimes accelerate it. Müller³⁶ also stated that high or toxic concentrations of glycosides decrease the resting potential, action potential, and action potential duration of cardiac muscles, while lower or nontoxic concentrations do not alter the resting potential. Similar stimulatory effects of this kind has been reported by several other investigators.⁴²⁻⁴⁸ In this connection, the positive inotropic effect of digitalis is attributable to inhibition of the Na-pump or to an action of digitalis on some other systems, and further, whether the positive inotropic effect of digitalis is related to its effect on the Na-pump alone has not been elucidated completely.

In the present study the amplitude and duration of action potential increased or remained unchanged at the lower concentration of ouabain, and decreased at the higher concentrations, in spite of progressive increase in contractile force. These results suggest that cardiac glycosides may seem to have, at least in lower concentrations, a "paradoxical" stimulatory effect on the transport of Na, and that the positive inotropic effect of ouabain depends upon its concentration and is not proportional to its inhibition of the Na-pump, although the Na and K membrane conductances have not been measured in this experiment. These findings also suggest that the positive inotropic effect of ouabain may be attributable to an increase in intensity of active state, since the rate of development of tension increased without any significant change in the time to peak tension.⁴⁹

When the muscle was exposed to solution of low K concentration and then cumulative doses of ouabain were added, contractile force became maximum at a lower concentration than in the media of control extracellular K solution. These results suggest that low extracellular K concen-

tration makes the preparation more sensitive to the glycoside effect.

In conclusion, the present study on a comparison of the electrophysiological characteristics in human and guinea pig cardiac muscles have not revealed particularly marked differences concerning the behavior of the transmembrane action potential and the contractile characteristics. These comparative studies would be available to apply several points in clinical cardiology.

SUMMARY

The transmembrane action potential and contractile force of isolated human atrial muscles which were obtained during cardiac surgery by using cardio-pulmonary by-pass technique were studied, compared with those of guinea pig left atrium.

Force-frequency curves showed a characteristic triphasic pattern, just as other mammalian atria. When extracellular calcium concentration was raised, percent change in contractile force was strengthened at high frequency of stimulation. When extracellular potassium concentration was reduced, contractile force became stronger and showed a monophasic pattern in response to increased frequency of stimulation.

In a solution of lower ouabain concentration, action potential leaved unchanged or showed larger amplitude and longer duration. In a solution of higher or "therapeutic" concentration of ouabain, the duration of action potential was shortened, and in its "toxic" concentration, marked decreases in amplitude and duration were induced.

The positive inotropic effect of ouabain was attributable to an increase in intensity of active state, because the rate of development of tension was increased without any significant change in the time to peak tension. When extracellular potassium concentration was reduced, contractile force became maximum at lower concentration of ouabain, and further increase of concentration induced ectopic impulse formation. In other words, atrial muscles became more sensitive to ouabain in a solution with decreased extracellular potassium concentration.

Acknowledgement

The author is indebted to Drs. Hideto Masaki, Yasuo Takeda, and other members of the cardiovascular unit of the First Department of Surgery of the Kyushu University Hospital for their continuing cooperation throughout the course of the work. Grateful acknowledgement is also made to Prof. Toshiyuki Yanase for his

careful review of the manuscript in this investigation and to Drs. Hiroto Mashiba, Morio Ito, and Makoto Arita for their kind advice and encouragement. I also wish to thank Dr. H. B. Hamilton for his editorial assistance.

REFERENCES

1. WOODBURY, J. W., LEE, J., BRADY, A. J., & MERENDINO, K. A.: Transmembrane potentials from the human heart. *Circulation Research* 5: 179, 1957.
2. TRAUTWEIN, W., KASSEBAUM, D. G., NELSON, R. M., & HECHT, H. H.: Electrophysiological study of human heart muscle. *Circulation Research* 10: 306, 1962.
3. SLEATOR, W. & De GUBAREFF, T.: Transmembrane action potentials and contractions of human atrial muscle. *Amer. J. Physiol.* 206: 1000, 1964.
4. PRASAD, K., SINGH, S., & CALLAGHAN, J. C.: Transmembrane potential and contraction of human heart in relation to potassium and ouabain. *Jap. Heart J.* 12: 290, 1971.
5. BROMBERGER-BARNEA, B., CALDINI, P., & WITTENSTEIN, G. J.: Transmembrane potentials of the normal and hypothermic human heart. *Circulation Research* 7: 138, 1959.
6. van DAM, R. T. & DURRER, D.: Excitability and electrical activity of human myocardial strip from the left atrial appendage in cases of rheumatic mitral stenosis. *Circulation Research* 9: 509, 1961.
7. LEVY, J. V.: Force-frequency relationship of human atrial muscle in vitro: Beta-adrenergic mechanisms. *Arch. int. Physiol. Biochem.* 76: 680, 1968.
8. SONNENBLICK, E. H., BRAUNWALD, E., & MORROW, A. G.: The contractile properties of human heart muscle: studies on myocardial mechanics of surgically excised papillary muscles. *Journal of clinical Investigation* 44: 966, 1965.
9. PRASAD, K. & CALLAGHAN, J. C.: Effect of replacement of potassium by rubidium on the transmembrane action potential and contractility of human papillary muscle. *Circulation Research* 14: 157, 1969.
10. TSIFUTIS, A., BURTON, R. M., & GOLDRING, D.: The effect of hypothermia and anoxia upon oxygen consumption and contractility of human and rat heart muscle. *Amer. Heart J.* 79: 88, 1970.
11. COLTART, D. J. & MELDRUM, S. J.: A comparison of the transmembrane action potential of the human and canine myocardium. *Cardiology* 55: 340, 1970.
12. FABIATO, A. & FABIATO, F.: The two components of the human atrial action potential. *Circulation Research* 19: 296, 1971.
13. GELBAND, H., BUSH, H. L., ROSEN, M. R., MYERBURG, R. J., & HOFFMAN, B. F.: Electrophysiologic properties of isolated preparations of human atrial myocardium. *Circulation Research* 30: 293, 1972.
14. FUJINO, T., FUKUSHIMA, I., TANOUE, M., SAEKI, K., ARITA, M., & MASHIBA, H.: Physiological studies on human cardiac muscle. *Jap. Circul. J.* 32: 1843 1968.
15. BLINKS, J. R.: Field stimulation as a means of effecting the graded release of autonomic transmitters in isolated heart muscle. *J. Pharmacol. Exp. Ther.* 151: 221, 1966.
16. BLINKS, J. R. & KOCH-WESER, J.: Analysis of the effects of changes in rate and rhythm upon myocardial contractility. *J. Pharmacol.* 134: 373, 1961.
17. KOCH-WESER, J. & BLINKS, J. R.: The influence of the interval between beats on myocardial contractility. *Pharma. Rev.* 15: 601, 1963.
18. STEIN, A. A., THIBODEAU, F., & STRANAHAN, A.: Electron microscopic studies of human myocardium. *J. Am. Med. Assoc.* 192: 139, 1962.
19. WEIDMANN, S.: Elektrophysiologie der Herzmuskel-fasern, Japanese translation by K. Matsuda. *Kinpo do publishing Co. Ltd.* Tokyo. 1957.
20. HOFFMAN, B. F. & CRANFIELD, P. F.: Electrophysiology of the heart. McGraw-Hill Book company, Inc. New York. 1960.
21. GIBBS, C. L. & JOHNSON, E. A.: Effect of changes in frequency of stimulation upon rabbit ventricular action potential. *Circulation Research* 9: 165, 1961.
22. HAJDU, S.: Mechanism of staircase and contraction in ventricular muscle. *Amer. J. Physiol.* 174: 371, 1953.
23. KATZUNG, B., ROSIN, H., & SCHEIDER, F.: Frequency-force relationship in the rabbit auricle and its modification by some metabolic inhibitors. *J. Pharmacol.* 120: 324, 1957.
24. KATZUNG, B. & FARAH, A.: Influence of temperature and rate on the contractility of isolated turtle myocardium. *Amer. J. Physiol.* 184: 557, 1956.
25. TUTTLE, R. S. & FARAH, A.: The effect of ouabain on the frequency-force relation and on post-stimulation potentiation in isolated atrial and ventricular muscle. *J. Pharmacol.* 135: 142, 1962.
26. KRUTA, V. & STEJSKALOVA, J.: Allure de la contractilité et fréquence optimale du myocarde auriculaire chez quelques mammifères. *Arch. int. Physiol.* 68: 52, 1960.
27. HOLLANDER, P. B. & WEBB, J. L.: Cellular membrane potentials and contractility of normal rat atrium and the effects of temperature, tension and stimulus frequency. *Circulation Research* 3: 604, 1955.
28. WINEGRAD, S. & SHANES, A. M.: Calcium flux and contractility in guinea pig atria. *J. Gen. Physiol.* 45: 371, 1962.
29. GROSSMAN, A. & FURCHGOTT, R. F.: The effects of frequency of stimulation and calcium concentration on Ca^{45} exchange and contractility on the isolated guinea pig auricle. *J. Pharmacol. Exp. Ther.* 143: 120, 1964.
30. SCHATZMAN, H. J.: Herglykoside als hemmstoffe für den activen Kalium und Natriumtransport durch die erythrocytenmembran. *Helv. Physiol. ACTA* 11: 346, 1953.
31. EDWARDS, C. & HARRIS, E. J.: Factors influencing the sodium movement in frog muscle with a discussion of the mechanism of sodium movement. *J. Physiol.* 135: 567, 1957.
32. TUTTLE, R. S., WITT, P. N., & FARAH, A.: The influence of ouabain on intracellular sodium and

- potassium concentrations in the rabbit myocardium. *J. Pharmacol. Exp. Ther.* 133: 281, 1961.
33. KAHN, J. B., EAKIN, E., & LEVI, D. E.: Effects of ouabain and calcium on potassium balance of isolated guinea pig ventricle. *Am. J. Physiol.* 203: 1130, 1962.
 34. KASSEBAUM, D. G.: Electrophysiological effects of strophanthin in the heart. *J. Pharmacol. Exp. Ther.* 140: 329, 1963.
 35. PAGE, E.: The actions of cardiac glycosides on heart muscle cells. *Circulation* 30: 237, 1964.
 36. MÜLLER, P.: Ouabain effects on cardiac contraction, action potential, and cellular potassium. *Circulation Research* 17: 46, 1965.
 37. TEN EICK, R. E. & HOFFMAN, B. F.: Chronotropic effect of cardiac glycosides in cats, dogs, and rabbits. *Circulation Research* 25: 365, 1969.
 38. ITO, M., HOLLANDER, P. B., MARKS, B. H., & DUTTA, S.: The effects of six cardiac glycosides on the transmembrane potential and contractile characteristics of the right ventricle of guinea pigs. *J. Pharmacol. Exp. Ther.* 172: 188, 1970.
 39. BRENNAN, F. J., McCANS, J. L., CHIONG, M. A., & PARKER J. O.: Effects of ouabain on myocardial potassium and sodium balance in man. *Circulation* 45: 107, 1972.
 40. FISCH, C. & SURAWICZ, B.: Digitalis. Grune & Stratton, Inc. New York. 1969.
 41. WILBRANDT, W. & WEISS, E. M.: Antagonism between cardiac glycosides and corticosteroids in the frog's skin potential. *Arzneimittelforsch.* 10: 409, 1960.
 42. MacROBBIE, E. A. C. & USSING, H. H.: Osmotic behaviour of the epithelial cells of frog skin. *Acta physiol. Scand.* 53: 348, 1961.
 43. McCLANE, T. K.: A biphasic action of ouabain on sodium transport in the toad bladder. *J. Pharmacol. Exp. Ther.* 148: 106, 1965.
 44. REPKE, K.: Metabolism of cardiac glycosides. Proc. 1st Int. Pharmacol. Meeting, Stockholm, 1961, Vol. 3, p. 47-73. Pergamon Press, Oxford, 1963.
 45. LEE, K. S. & YU, D. H.: A study of the sodium and potassium activated adenosine-triphosphatase activity of heart microsomal fraction. *Biochem. Pharmac.* 12: 1235, 1963.
 46. BONTING, S. L., HAWKINS, N. M., & CANADY, M. R.: Studies of sodium-potassium activated adenosine triphosphatase. *Biochem. Pharmac.* 13: 13, 1964.
 47. PALMER, R. F. & NECHAY, B. R.: Biphasic renal effects of ouabain in the chicken: correlation with a microsomal $\text{Na}^+ - \text{K}^+$ stimulated ATP-ase. *J. Pharmacol. Exp. Ther.* 146: 92, 1964.
 48. PALMER, R. F., LASSETER, K. C., & MELVIN, S. L.: Stimulation of Na^+ and K^+ dependent adenosine triphosphatase by ouabain. *Arch. Biochem.* 113: 629, 1966.
 49. SONNENBLICK, E. H.: Force-velocity relations in mammalian heart muscle. *Am. J. Physiol.* 205: 931, 1962.