Studies on the Mechanism of Cardiotonica with Reference to the Cardiac Glycoside, Calcium Ion and Myocardial Catecholamine in the Canine Heart

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With use of the myocardial tissue strip of the dog's left ventricle of the heart, the isometric contractility and poststimulation potentiation were measured. The studies were directed on the possible influence of calcium ion, potassium ion and the myocardial catecholamine on the positive inotropic action of the cardiac glycoside by employing the normal myocardial tissue piece. The studies were extended to the use of various types of the autonomic nervous blocking agent on the myocardium in which the action of catecholamine was actively suppressed, and also tried on the hypertrophic heart muscle strip removed from the dog developed of the aortic insufficiency.

It was concluded that the myocardial catecholamine exerts the regulatory function in the utilized process of the extracellular calcium ion relative to cardiac contraction in the normal and hypertrophic hearts.

CLINICAL application of the digitalis has been studied for years since its discovery by Withering. The pharmacologic investigation of the drug has also been extended to various aspects encompassing membranous potential, electrolytes, excitation-contraction coupling, myocontractile protein and energy metabolic process. Despite extensive search, however, its action in some respect seemed uncertain and still in the state of mystery as BING remarked¹.

With advanced studies made on the calcium factor, we are in better position to appreciate understanding of the muscular contraction and the mechanism involved. It has been presumed that by combining calcium with the contractile protein would initiate the excitation and contraction movement results;²⁻⁵ and it has been confirmed that basically, there was no difference to be observed whether the skeletal muscle or cardiac muscle as far as contraction process goes⁵. Nevertheless, it has been revealed that between the two types of muscle, certain dif-

ference exists, that the cardiac muscle is said to be particularly sensitive to the extracellular calcium⁵.

Since the report of LOEWI⁷, it has been called our attention to the similarity of action between the digitalis and calcium in reference to stimulative effect of the heart. The mode of action of calcium ion or how far calcium would take part in the digitalis effect is not clear, however, positive inotropic action (PIA) of digitalis apparently assumes to be based upon the mechanism of excitation-contraction coupling, as has been advocated⁸.

The digitalis has been known for years effective in abolishing the staircase phenomenon, while recent findings have supported a view that this phenomenon and poststimulation potentiation (PSP) undergo similar physiological process⁹ although FARAH¹⁰ has construed that PSP serves as an index for the excitation-contraction mechanism.

Digitalis in vivo is believed to possess enhancing action of the cardiac output in the

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failing heart which is not the case in normal heart. Accumulated evidences have shown that in the cardiac insufficiency, there are found not only the elevated blood catecholamine but its increased urinary output and the reduced myocardial catecholamine, creating a state of sympathetic tension^{11,12}.

TANZ¹³ found that the reason for digitalis to strengthen the contraction of heart could be attributed to release of the myocardial catecholamine and that maximum PIA would not occur when the catecholamine content remain low. SPANN et al.¹⁴, on the other hand, supported a view that presence of the hormones is least essential in the contraction of heart¹⁵, and that PIA of digitalis least affected by cardiac catecholamine despite its importance in vivo has been recognized¹².

In this investigation PIA of digitalis in vitro was studied by employing a circular muscle section of the dog's left ventricle of the heart and tried to show significance of the myocardial catecholamine from the aspect of PIA of digitalis related to extracellular calcium concentration and PSP variation in the heart tissue in which catecholamine activity was suppressed. The studies were extended to pathophysiological significance of catecholamine in cardiac failure and its relation to the action of digitalis.

METHOD

Healthy normal male dogs weighing 8.0 to 16.0 kg were subcutaneously administered of morphine hydrochloride 2 mg/kg followed by intravenous administration of sodium hexobarbital 40 mg/kg. The chest cavity was opened at the left 4th intercostal space and the thorax was exposed. The ascending aorta was first ligated and the beating heart was extirpated with the lungs; immediately, the left ventricle (1.5×4.0 cm) was resected parallel with the horizontal axis 1 cm below the ventricular outlet of the aorta. A medium circular muscle fiber was identified and dissected with the blunt end of the glass stick specially adapted. The excised heart tissue 20×0.4×0.4 mm was revived in the perfusion fluid by means of electric stimulation. The one end was fixed and the other end was connected with U-shaped strain gauge (Shinko Tsushin Company made) and continuously recorded of isometric contractile force with the Oscillograph WTR 211-2C of Watanabe Precision Instrument Company (Fig. 1).

The perfusate used composed of Na 149 mM, K

Fig. 1. Apparatus for measuring contractile force of heart muscle.

4.0 mM, Ca 2.0 mM, Mg 0.49 mM, dextrose 11.1 mM, pH 7.4, temperature $37.0 \pm 0.5^\circ\text{C}$, osmotic pressure 299 mOsm and saturated solution made up of mixed gases of 95% O_2 + 5% CO_2 . Po_2 in the perfusate was 302 mmHg, while Pco_2 was 16.7 mmHg.

For electrical stimulation a stimulator MSE type III of Japan Photoelectric Company was utilized with 5 mS short wave 30 times a minute, using slightly higher electric pressure than the threshold. Two groups of the dogs were used in reserpine administered experiment; the group consisted of normal male dogs was administered of 0.1 mg/kg intramuscularly in two times 48 hours and 24 hours before, while the other group of dogs had the same dosage once 24 hours prior to sacrifice.

The muscular section of the failing heart was obtained from the dog induced of the aortic insufficiency. The procedure followed was by making a perforation in the aortic valve having a diameter of 5 mm with the leaflet punching instrument which is encased in a steel tube with a diameter of 2.0 mm, and so devised that a scalpel like blade could be opened by the other end (Fig. 2). By inserting the

Fig. 2. The aortic leaflet punching instrument.
A) closed B) opened

instrument through the right carotid artery punching of the cusp of aortic valve is possible. It usually takes about three months to develop well established cardiac insufficiency which provided specimens to be used.

Quantitative determination of norepinephrine (NE) in the myocardium was taken by the modified THI method¹⁷ with the autorecording fluorescent spectrophotometer (MPF-2 type Hitachi) by making fluosensitive after extraction by the Crout's method¹⁶.

Determination of the poststimulation potentiation is based on the basal rate of 0.2/sec frequency of electrical stimulation, which follows 5/sec excitation for a period of five seconds and ten seconds rest period. The first contractile force was expressed in terms of added rate (100 per cent rate) against previous value of contractile force (Fig. 3).

$$\left(\frac{b-a}{a} \times 100 (\%) \right)$$

Fig. 3. Poststimulation potentiation obtained from the canine left ventricular heart strips. PSP was produced at a basal rate of 0.2 per sec, fast rate 5 per sec; rest period 10 sec.

Bethanidine sulfate (anti-releaser), and propranolol (beta-blocker) were employed as the sympathetico-toxic drug; these agents have been added to the perfusate to react on the heart muscle tissue.

RESULTS

I. METHODOLOGICAL APPRAISAL

(1) Influence of Temperature and Time (Fig. 4)

When the temperature of perfusion fluid reached below 34°C, the amplitude of isometric contraction would decrease, which indicates prolongation of duration and build-up time. When however, rose above 38°C, the amplitude would increase with slightly shortened duration and build-up time, in other word, the temperature range of 34°C to 38°C offers the most stable circumstance as far as amplitude, duration and build-up time are concerned.

While no stability of amplitude, duration and rhythmicity has been observed in the contractility of cardiac muscle tissue 30 minutes after reviving, these conditions soon began to show up stability in one hour and continue for

Fig. 4. Effects of temperature and time course on contractility.

a period of 70 minutes; and then noticeable decrease would occur and reached roughly 10 per cent after 100 minutes period but with no changes of duration and build-up time. This is the reason that the isometric contractility should be recorded during the stable phase. The electron microscopic observations of these cardiac tissue strips were made three hours after resuscitated condition in the perfusion fluid. It was observed that the cristae of the mitochondria could be clearly visible but no degenerative changes such as vacuolar formation or swelling to be recognized. Morphologically and numerically the glycogen granules showed no difference from the normal (Fig. 5).

(2) Concentration of G-Strophanthin (G-St.) (Fig. 6)

Pharmacologic effect of the digitalis has been known to vary considerably according to the species of animal. The examination of the dog's left myocardial tissue on the action of G-St. with varied dosage were tried. The Maximum Effective time (MET) of G-St. on 1.13×10^{-7} M was found to be 40 minutes. The amplitude of the contraction after 70 minutes began to decrease rapidly. By raising the used doses up

Fig. 5. Electron-microscopic finding obtained from the heart muscle which is beated in perfusate for 3 hours.

to $3.39 \times 10^{-7} \text{M}$, its duration could be shortened considerably. On the contrary, if the concentration of G-St. was lowered to $5.6 \times 10^{-8} \text{M}$, MET would have delayed. The positive inotropic effect of G-St. was shown to be as follows: with G-St. 5.56×10^{-8} , 1.13×10^{-7} , 2.26×10^{-7} and $3.39 \times 10^{-7} \text{M}$, these turn out to be +95%, +95%, +100% and 105% respectively. It seemed that difference due to variable concentration is less marked considering

Fig. 6. Contractile force obtained on the canine heart muscle following various doses of G-strophanthine as indicated, in terms of Mol.

the PIA, but taking MET and its duration into consideration, the optimum amount of G-St. to be added to the perfusate should be $1.13 \times 10^{-7} \text{M}$, and in this study, this concentration was adopted.

II. PIA OF G-STROPHANTHIN AND CALCIUM CONCENTRATION (Fig. 7)

To the perfusate, G-St. $1.13 \times 10^{-7} \text{M}$ was added every half hour and studies on PIA were made under various calcium concentration (1 mM, 2 mM, 4 mM) (Fig. 7). The myocardium react immediately with difference in the concentration of calcium; usually it only takes two minutes for the myocardium to adjust. If we were to make a standard for the myocardial contractility in the perfusate containing calcium concentration of 2 mM, with 4 mM, the increase would be 110 per cent. When lowered to 1 mM, the decrease would be 55 per cent. The PIA of G-St. on various Ca concentration was shown to be as follows: with Ca 4 mM, 2 mM and 1 mM, these turn out to be +15%, +125%, +147% respectively. However, MET were 25 minutes, 35 minutes and 110 minutes respectively. The dosage required for maximum

Fig. 7. Mean values of contractile force obtained in the canine heart muscle strips following G-strophanthine in perfusate of various calcium concentration.

↓ : G-strophanthine; $1.13 \times 10^{-7} M$

effect were $9.4 \times 10^{-8} M$, $1.32 \times 10^{-7} M$ and $3.77 \times 10^{-7} M$. This means that under the low calcium concentration, PIA is realized by increasing the dosage of G-St. to a larger extent. With appearance of the maximum effect of PIA for G-St., the condition becomes fixed and unrelated to Ca concentration in the perfusate, which supports the similarity of constancy of the maximal effect of Ca + G-St. in PIA regardless of Ca concentration.

III. RELATION OF G-STROPHANTHIN AND MYOCARDIAL CATECHOLAMINE

(1) Concentration of Calcium and Norepinephrine (NE)

PIA was observed (Fig. 8A) by making various strength of Ca in the perfusate (1mM, 2mM and 4mM) which were added of extrinsic NE 0.1 mcg/ml. Under the condition of 2mM Ca perfusate, PIA of NE responded markedly and showed increase of +70 per cent, which was no more different than with Ca 1mM. It appears that under such condition, NE had substituted for reduced contractility resulting from decreased Ca. Even with higher concentration of Ca 4mM, PIA of NE could still be detectable but lesser in degree as shown in 1mM or 2mM. When the catecholamine releaser—tyramine 10mcg/ml was utilized, the pattern of Ca concentration of 1mM or 2mM have shown the same type with the result of the extrinsic NE (Fig. 8B). While with higher Ca concentration of 4mM, PIA would be more

Fig. 8. Response of heart muscle to norepinephrine (0.1 mcg/ml) (A) and tyramine (10mcg/ml) (B) in perfusate of various Ca concentration.

marked than that of NE. Results have indicated that the myocardium is hyperreactive to catecholamine under lower Ca concentration. It is assumed then, that tyramine exerts mobilization of the catecholamine parallel with calcium concentration.

(2) PIA of G-St. and the Cardiac Catecholamine State.

It has been demonstrated that the canine heart muscle contraction could be fortified with previous treatment of bethanidine sulfate 3 mcg/ml (Fig. 9). This increase is transient in nature, decrease as much as 70 per cent would occur 120 minutes after the treatment and then remains fairly well fixed contractility. When G-St. $1.13 \times 10^{-7} M$ was administered to the heart muscle strip 60min. after bethanidine treatment, PIA turned to +110 per cent, which was no more different than that of G-St. effect of untreated muscular strip.

When however, the same dosage of G-St. was administered to the heart muscle 120 min. after bethanidine treatment, the PIA showed significant reduction of +35%. Yet, when the G-St.

Fig. 9. Effects of G-strophanthine on contractility of the canine heart muscle strips with prior treatment with bethanidine sulfate (3 mcg/ml).

dosage was increased up to 2.26×10^{-7} M, or 3.39×10^{-7} M, PIA rise was +74% and +112% respectively, though the effective period of time would be shortened. With the bethanidine treatment of three hours, NE 0.1 mcg/ml became strongly reactive compared with the untreated case. Tyramine effect with 10 mcg/ml was either lost or noticeably slow.

With propranolol treated strips (Fig. 10) in the increasing dosage of 0.1 mcg/ml, 0.5 mcg/ml and 1.0 mcg/ml, the contractility would be lowered and by the end of 30 minutes, the de-

Fig. 10. Effect of G-strophanthine on contractility of the canine heart muscle strips with prior treatment with various dose of propranolol.

crease occurred were 87%, 72% and 38% respectively. With G-St. 1.13×10^{-7} M, PIA values were +115%, +115% and +36% respectively, and with respective delay of MET of 70 minutes, 90 minutes and 105 minutes. PIA of G-St. would be markedly inhibited with propranolol 1.0 mcg/ml. With propranolol 0.5 mcg/ml treatment, PIA of G-St. 0.56×10^{-8} M showed +52%, while with 1.13×10^{-7} M, it was changed to +115%. Thus, increased drug dosage would cause proportionate decrease of PIA, and at the same delayed MET resulted. When treated with propranolol 0.5 mcg/ml, the muscle exhibited neither reaction with NE 0.1 mcg/ml, as well as tyramine 10 mcg/ml nor in a period of PIA with an addition of G-St.

With twice treatments of reserpine, it was found that the muscular tension of 0.022 g/mg wet weight seemed to have reduced two third compared with the normal heart strip (Fig. 11). With the group which received one treatment however, showed increase of 2.2 times, 0.073 g/mg wet weight. NE content of the heart muscle showed the decrease of 0.02 mcg/g, and 0.08 mcg/g. (Untreated group showed $1.0 \text{ mcg/g} \pm 0.3$). With the group treated twice showed the toxic symptoms characterized by the absence of response to stimulation, severe diarrhea and lowered body temperature, as WITHINGTON had observed in cat¹⁸. When the strips were treated with reserpine, G-St. would show +100%, +70% PIA respectively which is lower than the normal instance, this is more pronounced in the group had one treatment, but the MET of both groups were no significant

difference to be seen (Fig. 11).

IV. RELATIONSHIP BETWEEN PIA OF G-St. AND CATECHOLAMINE WITH REFERENCE TO CALCIUM CONCENTRATION

The effect of G-St. on the heart strip by changing calcium concentration in the perfusate under inhibited catecholamine action have been observed. When the muscular tissue was previously treated with bethanidine 3 mcg/ml for three hours in the low calcium perfusate (1 mM), the contractility resulted in 36 per cent

reduction when compared with calcium 2 mM concentration (Fig. 12-A). However, the change practically unnoticed when compared with untreated group (Fig. 7). PIA of G-St. with calcium concentration of 1 mM, 2 mM and 4 mM were +16%, +110% and 220% respectively. These figures mean inhibition of PIA would be most marked under low Ca concentration compared with the untreated group; on the contrary, when with higher concentration, it would show up the elevated curve. Upon the second

Fig. 11. Effect of G-strophanthine on contractility of the canine heart muscle with (B) and without (A) prior reserpinization.

a: Twice reserpinization 48 hours and 24 hours before.
b: Once reserpinization 24 hours before.

Fig. 12. Effect of G-strophanthine on contractility of the canine heart muscle with prior treatment with bethanidine (A) or reserpine (B) in perfusate of various Ca concentration.

↓: G-strophanthine 1.13×10^{-7} M

administration, PIA would attain the maximum elevation in each instance. The consumed time and dosage for possible effect of G-St. with reference to influence of calcium seemed to have no longer associated with delayed effective time or dosage to be increased.

By reserpinization with 0.1 mg/kg for 24 hours (Fig. 12-B) the contractility under high calcium (4 mM) showed +63% indicating to be lower than the control group, the figure followed that of bethanidine affecting PIA of G-St. under various concentration of calcium; that is, the lower concentration of calcium suppress the curve of PIA and the higher concentration became conspicuous curve. The effective time and the drug dosage are practically uninfluenced by calcium.

V. RELATIONSHIP BETWEEN PIA OF G-St. AND CARDIAC CATECHOLAMINE WITH REFERENCE TO POTASSIUM CONCENTRATION

Assuming isometric contraction of the normal

Fig. 13. Effect of G-strophanthine on contractility of the canine heart muscle, with and without reserpinization, in perfusate of various potassium concentration.

A) without reserpinization

B) with reserpinization

↓: G-strophanthine 1.13×10^{-7} M.

heart muscle strip in concentration (4 mM) of potassium as a standard amplitude, 2 mM and 8 mM would show respective figure of +65% and -20%. With 2 mM, 4 mM and 8 mM potassium concentration, G-St. PIA figures obtained were +60%, +104% and 74% respectively (Fig. 13-A). With higher potassium concentration, a longer time element would be required to obtain the maximal PIA and proportionate increased dosage as shown in low calcium concentration (Fig. 7). Low potassium implies rapid effectiveness of G-St. associated with accelerated automatism. By carrying out reserpinization of 0.1 mg/kg for 24 hours, the PIA in 2 mM and 8 mM in potassium concentration disclosed +64% and -17% respectively which was no more different than those untreated ones. PIA induced with G-St. then turned out to be +34%, +71% and +81% respectively at 2 mM, 4 mM and 8 mM of potassium. Under high potassium concentration, the effective dosage has to be increased whereas under low concentration, an automatism participates similar to the untreated ones which in turn implies the fact that variation of potassium in the perfusate failed to show any difference with PIA of G-St. on reserpinization animals.

VI. EVALUATION OF POSTSTIMULATION POTENTIATION

Fig. 14. Effects of G-strophanthine on contractility and poststimulation potentiation.

↓: G-strophanthine 1.13×10^{-7} M.

(PSP)

The PSP of the normal heart strip prior to G-St. administration was found to be $96 \pm 14\%$ which apparently showed proportionate decrease with relative increase of PIA of G-St. (Fig. 14). The PSP exhibited 10 per cent, when PIA reached at its maximum. The figure 15 is the variation of PSP by G-St., following treatment with various agents. Bethanidine treated group displayed higher PSP than the control strips, but with aortic insufficiency (AI) strips revealed the low figure. When administered of G-St. it presented low figure similar to the normal group, and with AI strip, the figure would be 20 per cent lower always with elapse of time.

Correlation of G-St. effect on increase of contractility rate and PSP prior to drug administration in the normal dog's heart was shown in Fig. 16. With calcium 2 mM in perfusate, above correlation could be clearly demonstrated, whereas in the condition of calcium in 4 mM, PSP as well as PIA both were reduced, hence regardless of calcium concentration, significant correlation does exist between the two, with risk rate of 1% and correlation coefficient $r=0.825$. If PSP showed -6% prior to G-St. administration, no PIA of either G-St. or calcium

Fig. 15. Effect of G-strophanthine on poststimulation potentiation of the canine heart muscles with or without prior treatment of various drugs.

could be observed, thus it is assumed that there occurs close relationship recognizable between

Fig. 16. Correlation of effects of G-strophanthine on contractility and poststimulation potentiation in the canine heart muscle.

Fig. 17. Correlation of effects of G-strophanthine on contractility and poststimulation potentiation in the canine heart muscle with prior treatment with reserpine (A) or bethanidine (B).

PSP and PIA of G-St. With bethanidine and reserpine trials (Figs. 17-A & 17-B), correlation between increased rate of contractility with G-St. and PSP before administration of G-St. showed coefficient $\gamma = -0.190$ and 0.164 , which simply means no obvious correlation existed. Reserpinized or bethanidinized muscle piece disclosed the reduction rate of PSP in small degree even with concentration of calcium raised, which speaks for marked difference from normal group.

VII. RESULTS ON THE AORTIC VALVE INSUFFICIENCY DOG (AI Dog)

Those dogs experimentally produced of the aortic insufficiency survived for a period of 90 days and the conditions found at the time of death were shown in the Table I. The heart weighed 102 ± 21.8 g while the weight of lung was 145 ± 20 g showing increase in comparison with the controls. The heart-body weight index and lung-body weight index showed $+26\%$ and $+47\%$ increase respectively, while the lung-heart index had $+16\%$ increase.

NE content of the cardiac muscle in the control group was found to be 1.0 ± 0.3 mcg/g wet weight, but in the AI heart 0.38 ± 0.19 mcg/g wet weight. As shown in the coefficients, AI animal definitely demonstrated the heart load which eventuated to the left cardiac hypertrophy. The contractility (PIA of G-St.) variations due to changes in concentration of calcium in the perfusate were: with calcium concentration 1 mM, and 4 mM, -47% and $+111\%$ respectively, which also proved no difference compared with the control (Fig. 18). With calcium concentration of 2 mM, PIA of G-St. 1.13×10^{-7} M was $+84\%$ showing slight decrease from the control animals and the initial contractile tension showed two fold of the controls, that is 0.062 g/mg wet weight (Fig. 18). With low calcium (1 mM), PIA of G-St. was $+18\%$ indicating markedly lessening from the controls. Under the condition of calcium (4 mM), PIA

showed marked elevation of $+182\%$. Influence of calcium for the maximum effective time in the control heart could not be identified. It appears that relationship between calcium and PIA found in the AI heart closely follows those of reserpinized or bethanidinized.

The figure 19 shows the correlation of pre-administrative PSP value and PIA of G-St. and the coefficient of Ca 2 mM was $\gamma = 0.804$ with 1% risk rate. This correlated figures are significant. With relatively high Ca perfusate (4 mM), PSP came down sharply as seen in the normal specimens (Fig. 16), but definitely indicating PIA of G-St. is different from those of the controls. Relation between PSP and PIA in AI heart muscle does not seem to coincide because of calcium concentration, that is, the higher concentration of calcium becomes, the more shift occurs toward the right.

Fig. 18. Effects of G-strophanthine on contractility in the canine heart muscle with aortic insufficiency in perfusate of various Ca concentration. $\uparrow$: G-strophanthine 1.13×10^{-7} M

TABLE I CONDITIONS OF DOGS WITH AORTIC INSUFFICIENCY

	Body Weight (kg)	Heart Weight (g)	Lung Weight (g)	Heart-Body Index (g/kg)	Lung-Body Index (g/kg)	Lung-Heart Index
Control	10.5 ± 1.9	79 ± 14.4	96 ± 13	7.50 ± 0.37	9.10 ± 1.27	1.2 ± 0.15
AI	10.8 ± 1.4	102 ± 21.8	145 ± 20	9.45 ± 0.66	13.4 ± 2.16	1.42 ± 0.33

Fig. 19. Correlation of effects of G-strophanthine on contractility and poststimulation potentiation in the canine heart muscle with aortic insufficiency in various Ca concentration.
 × Ca 1 mM, ● Ca 2 mM, ○ Ca 4 mM.

DISCUSSION

In the experiment *in vitro*, the auricular muscle tissue has been employed in most occasions. When the ventricular muscle piece is selected, the papillary muscle or the right ventricular tissue has been adopted commonly. The major function of the heart is known to be its pumping effect which is fulfilled by the left ventricle, consequently, it stands reason that the left ventricular muscle is the most suitable medium for the study of myocardium *in vitro*. For above reason, the annular muscle of the left ventricle was selected. Anatomically speaking, the left ventricular muscle is composed of unique tissue arrangement unlike the skeletal muscle fiber, for its distal end of each fiber is morphologically indistinguishable and its ending is not clearly visible, that is to say, these fibers are joined together in a lattice-work; in fact, the heart is composed of a muscle syncytium, which makes difficult to remove muscle tissue piece by separating the left ventricle. Awaiting this fact, the author has devised a special glass stick having a blunt end which make it possible to take the annular muscular piece of the dog with relative ease.

According to LEE¹⁹, for the purpose of keeping the sufficient diffusion of O₂ to the cylinder like muscle fiber which is immersed in the perfusate bubbled with 100 per cent oxygen, it should be of a diameter less than 1.2 mm, hav-

ing cross section of roughly 1.13 mm². The heart muscle tissue used for the present study had a cross section of roughly 0.5 mm² and well adopted for O₂ diffusion, in fact, it was possible to record stabilized contractility for a period of 70 minutes when resuscitated in the perfusion liquid and electrically stimulated to contract, indicating that the procedure has enabled to revive the metabolic process of the myocardium due to the proper character of the perfusate. Electron microscopy has already substantiated histologically on this point. The annular heart muscle tissue removed from the canine left ventricle seemed to have served the purpose similar to the auricular tissue or the papillary muscle.

Because sensitivity to digitalis varies with the species of animal, strength of G-St. had to be changed accordingly, as CHEN²⁰ had reported that the sensitivity for ouabain by the animal species were in the order of cat, rabbit, guinea pig, mouse and rat, or 1:2:2:62:671. Inotropic action may be opposed entirely positive or negative, according to concentration or duration as far as digitalis is concerned. Whether the used concentration exerts the therapeutic effect or toxic outcome should be judged. Recordings made of the curves on the reaction time of the ventricular muscle tissue under various concentration revealed the fact that these results coincided in general with the case of the cat's papillary muscle as reported by TANZ¹³, but the dog's tissue showed 1.5 time sensitive.

With the concentration of G-St. (5.51×10^{-8} M to 3.39×10^{-7} M), the greatest PIA was shown to be around 210% in each instance. The fact that increased concentration of G-St. would shorten MET does seem to agree with results of MORAN and KOCH-WESER^{21,22}. It is interpreted that the action of digitalis is contraction-dependent cumulative action, or frequency-dependent. Availability of PIA meant accumulative effect of digitalis or the certain substance mobilized by digitalis in the cell undergoing contraction. When these substances reached beyond certain level of concentration, PIA would likely be to disappear and became the state of contracture. PIA exhibits during the therapeutic phase anyway regardless of dosage, and the negative inotropic time meant the toxic phase which contradicts the results of

TUTTLE²³ who deduced from force-frequency curve. They opined that under the therapeutic dosage normalization was attained of force-frequency curve and PSP was greatly increased whereas under toxic dosage PSP decreased. With dog's heart muscle, PIA of G-St. had increased with simultaneous drop of PSP (Fig. 14). When PSP was unchanged, no PIA could be seen. When digitalis was administered to the case of cardiac insufficiency, increased cardiac output is one of the expected effect signifying increased force of contractility, in fact, it was proved that the digitalis is possessed of strengthening force of the heart even for the patient who is devoid of heart insufficiency²⁴. Therefore, whenever contractility is accelerated despite dosage consideration could easily be assumed as the therapeutic phase.

If perfusate lacking calcium, muscular contraction would no longer result but the cardiac muscle is equipped with most sensitive reaction unlike the voluntary muscle. LEONARD²⁵ and NAYLER²⁶ stated that in the cardiac tissue extracellular calcium would enter intracellular space during membranous excitation period and participate in the mechanism of contraction. It is essential that the extracellular Ca^{++} is indispensable in the function of contraction. With Ca^{45} trial, the digitalis showed increased Ca^{45} -uptake^{26,27} and PIA could readily be influenced by extracellular concentration of Ca. With rise of Ca, PIA was lowered, while, under low condition, it became higher²⁸⁻³⁰ which seemed to agree with the author's result.

With low Ca concentration, PIA would rise by increasing the dosage of G-St. and supplant the reduced contractility under low Ca of the extracellular space (Fig. 7). MET is prolonged under low Ca but shortened under higher condition, this also indicates the important role of Ca for accumulative digitalis action previously mentioned. This implies that by changing potassium of perfusate to higher concentration, PIA of digitalis similar to low Ca, brings for the prologation of MET and an increased drug dosage. This does point out a fact that one of the most effective site of digitalis would be strongly responsive to the extracellular Ca and K which is located in the neighborhood of cytoplasmic membrane. The synergistic action of

digitalis and calcium to fortify contractility meant also their similarity of pharmacologic action. Nonetheless, concerning potassium, before maximum PIA appears in the condition of low potassium, cardiac muscle was in the state of hyperautomatism, which seemed to account for antagonism of K ion and digitalis from a consideration of arrhythmic action as opposed to inotropic action.

It is a known fact that aside from digitalis, catecholamine is capable of influencing positive PIA and much has been elucidated from the aspects of metabolism^{31,32} and contractile mechanism^{33,34}. TANZ^{13,35} has asserted that the myocardial catecholamine is utterly indispensable for positive manifestation of PIA of digitalis, and advanced a hypotheses that the action of digitalis is to expedite to release the myocardial hormone which in turn induces PIA. The other experimental reports have supported his proposition³⁶. Against this, there have been a number of reports^{15,37,38} suggesting that the effective PIA by digitalis is possible to be observed under the condition of blocking the endocrine action by employing the suppressing agents of catecholamine. It was disclosed in the present studies that when bethanidine was used PIA of G-St. seemed to have been interfered as time went on, and for the maximum effect, the dosage needs to be increased, while propranolol which is beta-blocker is found to cause delay in the maximal effective time, and full PIA would not occur with its higher concentration. So that, it may be probable that PIA is the result of releasing of the hormone solely due to digitalis as TANZ asserted. With propranolol treated tissue, however, expected PIA has failed to demonstrate even reacted with NE or tyramine in the manifesting phase of PIA with G-St. and then PIA of G-St. had occurred in reserpinized tissue strip in the condition of catecholamine depletion. The similar reactive pattern was presented though the reactivity was much lesser in degree than control. With reserpinized tissue, catecholamine was found to be less than as much as 2 per cent of the normal level for which TANZ has proposed to be sufficient to prevent emerging PIA of digitalis; TANZ's hypothesis is hardly acceptable. The above fact in no way supports an interpretation that PIA

of G-St. is due to releasing of the cardiac hormone. As proposed by SPANN¹⁵ that the reason for PIA inhibition by the antiadrenergic drug was due to some extraneous factor other than the inherent antiadrenergic action of these agents should be supported. As to the synergism of calcium with digitalis the cardiac muscle exhibited hypersensitivity against NE in low strength of calcium, this is, however, more pronounced when NE is released by tyramine in higher Ca concentration which made it difficult to explain the synergism of G-St. with calcium from a standpoint of catecholamine discharge. The reason why Tanz's work did not show PIA, in which the papillary muscle of cat's reserpinized right ventricle was used, may be explained by the difference in the animal species and probably due to variation in the composition of the perfusate particularly of Ca, K and Na.

This raises some question about what a role of the cardiac catecholamine participates in the inotropic action of digitalis. SPANN stressed that it does not constitute an essential factor in the mechanism of contraction^{10,14}, while LEE³⁹ contradicted by saying it has an important role in the performance of normal contraction. The composition of electrolytes in the perfusate need to be considered in as much as their methods and materials used between the above two were not identical. The reason for this assumption was based on the result obtained that intimate relationship existed between PIA and calcium concentration, and when catecholamine activity was suppressed either by reserpinization or bethanidinization, no possible control of PIA of G-St. would occur by changing concentration of calcium in the perfusate (Fig. 12). Under high calcium concentration PIA of G-St. displayed little suppression but under low condition, almost identical increase in PIA seen in the former. With the bethanidine or reserpine, however, MET of PIA or dosage difference is no longer present either high or low calcium strength, meaning no synergism between digitalis and calcium observed as far as the control is concerned. On the other hand, with potassium concentration, no marked difference could be observed in PIA between the control and catecholamine suppressed muscle. Thus in the sup-

pressed state of catecholamine, PIA presents different attitude from the controls due to extracellular calcium concentration and no effect to be seen with extracellular potassium. It appears that catecholamine influences PIA through extracellular calcium, in other words, catecholamine is most necessary for synergism between PIA and extracellular calcium. Further discussion follows on the results which supports this assumption.

That so called "Staircase phenomenon of muscle" is bound to be lost upon the use of digitalis has been well known for years. A number of recent reports state that this mechanism involved in the staircase phenomenon proved to be identical with the condition of poststimulation potentiation^{9,29,40}. WAHLEN⁴¹ assumed however, that these phenomena were responsible to discharged myocardial catecholamine. To this some authors have expressed their disagreement^{14,42}. NAYLER⁴³ thought that PSP was unrelated with catecholamine release and that it is the result of repeated muscular contraction which caused the extracellular calcium to enter into the cells leading to intracellular accumulation which in turn assist succeeding contraction serving the chief factor for PSP. ROSIN⁹ deduced along this concept but the potentiation substance would be produced in the cells and accumulated which is in turn utilized for contraction force.

In this connection, FARAH²⁹ stated that the potentiating substance is identical with the activator concept proposed by NIEDERGERKE. All these men interpreted that PSP could have been produced by the potentiating substance (may be Ca or activator) causing contraction and affected by the action of digitalis and extracellular calcium concentration. As KATZUNG⁴⁵ reported on the use of metabolic poisoning, the potentiating phenomenon is a different process aside from energy production system. Hence, phenomenon of PSP should indicate excitation-contraction coupling as FARAH's²⁹ emphasis on PSP phenomenon. As shown in the results, PSP could be greatly influenced by external calcium concentration whereas it is less affected with the cardiac catecholamine, and PSP can be reduced by the administration of digitalis (Fig. 15, 16 and 17). These data have endorsed

the fact that above activator theory could be applied to the annular muscle tissue taken from the dog. Figure 16 shows significant relationship of PIA and PSP in the normal heart which refers to utilization of the available activator (R) in PIA of digitalis. The activator proposed by NIEDERGERKE could be inhibited by Na and K and made reactive by Ca. Assuming these facts, influence of Ca on PSP and correlation of digitalis and Ca as well as relationship of digitalis and potassium in the normal heart muscle might be readily explained. Under suppressed catecholamine activity due to either reserpinized or bethanidinized condition, PSP would be lost along with treated time of G-St. indicating no more difference from the normal heart (Fig. 15). Nevertheless, PSP and PIA showed $r = -0.190$ and 0.164 , hardly any relation to be observed (Fig. 17). As seen under low Ca concentration, though PSP is well maintained in the muscle piece, PIA was much reduced while under high calcium concentration PSP showed no reduction and PIA was definitely increased. The finding that PIA develops solely through the medium of activator could hardly be acceptable under suppressed catecholamine activity. It is strongly dependent on the process which would utilize directly the extracellular calcium. Besides the use of activator in the perfusate at Ca 2 mM and 4 mM (loss of PSP by G-St.), the cardiac muscle with non-active catecholamine probably can attain PIA by the use of external calcium ion without the medium of activator. Under 1 mM condition, it is assumed that PIA is liable to be lowered because of not only disturbed utilization of activator but also lowered extracellular calcium. It is explained that losing of the difference of MET of PIA and the therapeutic dosage in variable calcium strength is dependant upon the extracellular calcium rather than the activator under catecholamine inhibition. In opposition to this, the other explanation would be that catecholamine is necessary for the production of activator for PIA and to accomplish an important role in regulating cardiac contraction by external calcium ion, and also by controlling extra and intracellular calcium ions.

Although the significance of cardiac catecholamine has been stressed, reduction in the car-

diac insufficiency has also been reported. Hence, further investigation was attempted into the factor concerned in the reduction of catecholamine in the cardiac insufficiency and its influence on the contractile force. The reports on experimental congestive heart failure were many thus far but most of these trials were artificially induced stenosis of the pulmonary artery and the tricuspid valve insufficiency^{46,47}. These methods seem to offer secondary blood stasis as the result of circulatory disturbance of the right heart rather than creating an important manifestation of the decreased contractility. In view of these situations, in the present study, an attention was paid to the heart failure ascribable to loading on the left side. It has also been called attention on the experimental hypertrophic heart developed following the aortic stenosis as far as small animals such as the rat and rabbit were concerned^{48,49}. The author has attempted various experiments with the dogs but had to give up making experimental aortic stenosis dogs because of serious surgical stress involved in the thoracic surgery, and not infrequently caused rupture of the arterial wall at the stenosed area around 20th postoperative day which made difficult to keep survival of the animals, hence, the above described method of the aortic valve insufficiency has been tried. This procedure entails less surgical risk with relative ease of technique, compared with the method of NOLAN and ROSCHE^{50,51}, because the carotid artery was used. Another advantage is that the desired size of the valvular perforation could be produced. The hypertrophic heart is possible in AI dog employed in this trial similar to the case of the guinea pig with aortic stenosis⁴⁸. Significant reduction of the cardiac catecholamine can be taken as the stage of the insufficiency. Compared with the normal heart, AI heart seemed to increase the contractility during the first stage, attributed to the hypertrophy and extension of the muscle fibers⁵², which corresponds with the rising segment of Starling's curve. During the compensatory period, marked PIA was obtainable with the hypertrophic heart tissue strip but much less degree than the case of normal heart. With the hypertrophic heart, lowering of calcium in the perfusate would tend to reduce PIA while

elevation of calcium would have caused pronounced increased PIA compared with the normal heart. Relationship of PSP and increased rate of PIA showed coefficient $\gamma=0.804$ in Ca 2 mM, while with reduced PSP under high Ca condition showed hardly any difference from the normal, yet PIA of G-St. showed marked increase (Fig. 19). Above findings could hardly be explained from the concept of activator, for under the condition of high calcium content, PSP has already lost with well exhibited PIA despite no presence of activator. While under low Ca condition, G-St. does not seem to compensate the decreased contractility seen in the normal despite PSP still present. This proved to show that in the hypertrophic heart G-St. is fully dependant on the activator in the perfusate having Ca 1 mM with unsatisfactory utilization of extracellular calcium, but PIA would not show up sufficiently due to lowered Ca concentration. With Ca 4 mM, there are two approaches to attain PIA, one is to utilize activator and the other is to use extracellular calcium directly to contraction mechanism. The reason for losing difference in MET of G-St. due to variable calcium concentration may imply that in the normal heart, PIA could be exhibited only through production of activator, while with the hypertrophic heart, through the two process cited above, the time difference would seem to have been lost. The question is how this occurs in the hypertrophic heart. It is our interest to know that the result obtained from the hypertrophic heart and the data shown with reserpinized or bethanidinized heart presented some similarity. As a matter of fact in the experimental failing heart^{11,48} or the patient with insufficient heart, both reportedly showed lower myocardial catecholamine. Such reduction was also observed in AI dog, as much as 38 per cent. Much has been discussed up to present on the pathophysiologic significance of catecholamine referring to cardiac failure. Nevertheless, no fixed theory has been advanced. It has been reported that enhanced cardiac contractility could be responsible to catecholamine released by sympathetic stimulation or tyramine⁴², and the liberation of myocardial catecholamine by reserpine would increase the glycogen content of the myocardium

which in turn result in disturbed energy production. Importance of catecholamine is well explained in these reports, as shown previously, it is equally significant in the PIA of G-St. in the normal heart. In the hypertrophic heart, catecholamine reduction is much less compared with reserpinized case. But, there are two types of catecholamine, namely, available and bound types. Reserpine seemed to release the two types, while the sympathetic stimulation discharge available type⁵⁴. It is assumed therefore, decreased catecholamine in cardiac insufficiency is attributed to increased tension of the sympathetic system^{11,12,47}, that is, dependant on available type. Hence, in the condition of hypertrophy, cardiac function is greatly affected even though catecholamine decrease is only slight degree. Thus difference of PIA due to variable calcium concentration seen in the hypertrophic heart is presumably due to decreased catecholamine in the myocardium. Basing on these findings, PIA of G-St. act mainly through the extracellular calcium in the enlarged heart and the chief cause may be attributed to the decreased condition of cardiac catecholamine.

CONCLUSION

1. The isometric contraction of the heart was measured by employing the annular muscular tissue of the left ventricle of the dog's heart. The muscular strip has proved its merit comparable to the other part of the cardiac muscle.
2. It was well demonstrated that the canine ventricular muscle strip was found to be more sensitive to G-strophanthin, 1.5 fold than the case of papillary muscular tissue of the cat's heart. By taking the therapeutic phase as the time for PIA to show, then, 1.13×10^{-7} M was proved to be the optimum experimental concentration.
3. With the normal heart muscle tissue, PIA of G-St. exhibited synergism with calcium and presented the similarity of biochemical interaction.
4. The mechanism for PIA of G-St. was least involved the process of releasing cardiac catecholamine by G-St. and synergism of G-St. and calcium could hardly be explained from the catecholamine release.

5. Under the condition of suppressed catecholamine activity, the synergistic action of PIA of G-St. and calcium ion could not be detected and the influence of calcium for the effective time and dosage seemed to have lost, while effect of potassium ion was the same as found in the normal case.

6. Assuming the action of G-St. in the light of liberating activator in reference to poststimulation potentiation, participating activator evolved would have been disturbed under the condition of catecholamine inhibition. PIA could be elucidated mainly from the utilizable extracellular calcium in the performance of cardiac contraction. It was construed that for PIA to be effective, catecholamine plays an important role in the discharge of activator and utilization of the calcium.

7. No synergism between G-St. and calcium was observed in the hypertrophic heart of the dog suffering from cardiac failure due to aortic valvular insufficiency. Catecholamine decrease had reached to 38 per cent.

8. From the results obtained from poststimulation potentiation PIA of G-St. in the hypertrophic heart muscle strip was elicited mainly by utilizing extracellular calcium which was presumably due to decrease in the available fraction of catecholamine.

9. From the above results, the cardiac catecholamine is possessed of ability to regulate extracellular calcium involved in the cardiac contraction, whether the normal heart or failing heart.

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