The Tissue Catecholamine Concentration of the Rabbit in Experimental Cardiac Failure

YOSHIHISA ITO

The determination of urine and tissue catecholamine levels were undertaken in rabbit with or without cardiac failure produced by aortic obstruction. The tissue catecholamine have tended to be lowered in cardiac failure, especially in the heart, while urinary catecholamine levels showed elevation. Uptake of the exogenous noradrenaline by the heart was lowered in cardiac failure than that of the normal subject. It was found that with nialamide, propranolol, or bethanidine, catecholamine concentration of heart could have increased in normal health, but in cardiac failure, such increase could be suppressed. It has been known that a condition of catecholamine depletion of the tissues by reserpine appears to be associated with aggravation of cardiac failure.

The failing heart seems to yield acceleration of the sympathoadrenal activity, which results in depletion of tissue catecholamine perhaps due to the increased release of stored catecholamine and deficient retention by enhanced turnover rate, or lowered uptake in the tissues. It is concluded that the tissue catecholamine plays a compensatory role in cardiac failure.

THE AUTONOMIC nervous system plays an important role in the regulation of the body function, particularly in the cardiovascular performance. In order to study the pathophysiological significance of sympathetic nervous system in cardiac failure, it is necessary to observe the sympathoadrenal activity in the various circulatory disturbances. Although the methods for chemical determination of catecholamine (CA) have been established and clarified of metabolic pathway, further informations on the CA levels of urine, plasma and tissue in cardiac failure need to be advanced. Increased sympathoadrenal activity in cardiac failure by measuring the heightened urinary excretion of CA, changes of clinical findings and hemodynamics and the responses to the various autonomic blocking agents have been reported by Matsumoto, while RAAB has demonstrated marked elevation of CA in the myocardial tissue in acute heart failure, whereas BLOOMWORTH and von HAAM have failed to detect any signs of change in the total CA content of the failing human heart. CHIDSEY et al. on the other hand found a decrease in the noradrenaline content of the failing heart in their serial studies. There have been diversity of opinions relative to CA concentration in cardiac failure.

The present study is undertaken to evaluate the changes of CA and its pathophysiological significance in the cardiac failure by measuring urinary and tissue CA in the rabbit with or without experimental cardiac failure.

METHODS AND MATERIALS

The experimental animals; Albino rabbits of either sex, weighing 2 to 3 kg were used in all the experiments. The anesthetic agent employed was urethane (1g/kg), injected intraperitoneally. As shown in Fig. 1, for causing cardiac failure, the technique of aortic obstruction modified by UEBA-Oda

1 Department of Internal Medicine, First Division (Director: Prof. T. Tomomatsu) School of Medicine, Kobe University, Kobe
(Received for publication, March 4, 1968)

Japanese Circulation Journal Vol. 32, May 1968 761
was employed. A small piece of laminallia which was fixed at the tip of the vinyl tube (2 mm in diameter), was inserted into the right carotid artery and fixed just above the aortic valve. The laminallia expanded rapidly when inserted and was possible to produce the aortic obstruction in 20 to 30 minutes after introduced. The extent of pulmonary edema and the degree of cardiac hypertrophy were evaluated by the macroscopic and histological findings of the lung, the weight ratios of Lung/Body, Lung/Heart and Heart/Body. The sham method followed the similar procedure but with the use of vinyl tube without laminallia to be inserted into the carotid artery.

Estimation of urine and tissue CA: Specimens of urine were collected several days before surgery until the death, and 24-hour urine was acidulated with sulphric acid. Urinary CA was determined by the modified method of Imaiizumi-Nakajima4, absorbed by the aluminum oxide at pH 8.4 and extracted with acetic acid. After oxidizing trihydroxyindole (THI) with potassium ferricyanide, CA was determined fluorometrically12. The rabbits developed of failing heart by means of aortic obstruction were sacrificed by air embolism in 2 to 3 hours, 1, 2, 5, 9, 11 and 40 days after surgical procedure, and removed the heart and other organs immediately, these were weighed after removing wet. The heart was separated into three parts, the atria, left and right ventricles, leaving the ventricular septum to the left ventricle. Extraction of tissue CA was made by means of Crout's method10, by having homogenate prepared with a ground glass tissue homogenizer in about ten volumes of tissue weight or 20 cc of cold 5 per cent of trichloroacetic acid, and allowed to stand over 24 hours at 4°C and centrifuge for 20 minutes at 20,000 Xg. Collect the supernatant fluid and reextract the residue with cold 5 per cent of trichloroacetic acid. The combined extracts were used for estimation, based on the recovery rate which is obtainable by adding the fixed amount of noradrenalin or adrenalin to a part of the extracts. Then, 0.5 cc of 0.2 M 2Na-ethylendiaminetetraacetate (EDTA), 10 cc of 0.2 M sodium acetate and 0.5 g aluminum oxide (Merck's alumina, nonalkaline) were added to the extracts and the mixtures were adjusted to pH 8.4 by

![Diagram of the aortic obstruction technique](Image)

**Fig. 1. The technique of aortic obstruction.**

A small piece of laminallia, which was fixed at the tip of the vinyl tube (2 mm in diameter) (left figure) was inserted into the right carotid artery and positioned just above the aortic valve. After insertion the laminallia expanded and the aortic obstruction was produced (right figure)

<table>
<thead>
<tr>
<th>Table I</th>
<th>Analysis of Adrenaline and Noradrenaline in Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eluate (cc)</td>
</tr>
<tr>
<td>Sample</td>
<td>1.0</td>
</tr>
<tr>
<td>Sample blank</td>
<td>1.0</td>
</tr>
<tr>
<td>Ad, intern. stand.</td>
<td>1.0</td>
</tr>
<tr>
<td>NA, intern. stand.</td>
<td>1.0</td>
</tr>
<tr>
<td>Sample for recovery</td>
<td>1.0</td>
</tr>
<tr>
<td>Sample blank for recovery</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*: Adrenaline standard sol., containing 1 mcg adrenaline base per cc.
**: Noradrenaline standard sol., containing 1 mcg noradrenaline base per cc.
***: Eluate is adjusted to pH 6.0 by adding of 5% sodium bicarbonate.
****: A mixture of 9 parts of 5N sodium hydroxide and 1 part of a per cent solution of ascorbic acid.

adding 4N ammonium hydroxide per drop and stirred vigorously for 5 minutes. The alumina was allowed to settle and the supernatant fluid was decanted. By adding a few cc of 0.2M sodium acetate and transferred to a glass column 6mm in diameter with supporting disc at its lower end. The column was rinsed with 10cc of distilled water, extracted CA from the column with 4.8 cc of 0.2N acetic acid, exerting pressure to complete extraction by 15 to 20 minutes. To the extract 0.1cc of 0.2 M 2Na-EDTA was added, the total volume of 5cc was made by diluting with 0.2N acetic acid, and stored in frozen state at $-25^\circ$C. As Table I indicates CA was analysed by the modified method of Bertler et al. Adrenaline and noradrenaline values were determined fluorometrically by activating wave lengths of 410 and 455 m $\mu$ and fluorescent wave lengths of 510 m $\mu$ by using the Hitachi MPF-2 spectrophotofluorometer. The recovery rate of added adrenaline to the extract averaged 80.0 $\pm$ 5.5%, while noradrenaline averaged 73.0 $\pm$ 5.2%. The tissue CA values were corrected taking into consideration of recovery rate and reported in microgram per gram (mcg/g) wet tissue weight $\pm$ S.D. Excluding the adrenal gland, the adrenaline concentration of the various tissues were shown to be very low, especially in the heart, actually less than 3% per cent of noradrenaline. In the cardiac failure, CA concentration has shown the fact that the adrenaline value is practically unchanged, hence, only the noradrenaline had to be estimated for the tissue CA. In the rabbit, however, majority of CA in the adrenal gland composed of adrenaline, so that its concentration had to be estimated for CA concentration.

The following drugs were used in the studies: levarterenol (1-noradrenaline) bitartrate, nialamide, propranolol, bethanidine sulfate and reserpine phosphate. The dosage and time schedules are referred to the appropriate section of the results.

RESULTS

1) Preparation of Experimental Aortic Obstruction in the Rabbit:
Following the method of Ueba-Oda a piece of laminallia was inserted as far as proximal to the aortic valve. Pronounced pulmonary congestion was observed and proved by the histological finding which was macroscopically confirmed to III to IV grade of Jordan's classification. The Lung/Body weight ratio seemed to have significantly increased from 3.01 $\pm$ 0.19 of normal group to 6.29 $\pm$ 0.63; the Heart/Body weight ratio showed from 1.80 $\pm$ 0.11 to 3.02 $\pm$ 0.21, while, the Lung/Heart ratio rose from 1.70 $\pm$ 0.24 to 2.27 $\pm$ 0.45, which characterized the dilatation and hypertrophy of the heart. With the sham operative procedure group, having introduced of the straight vinyl tube showed the Lung/Body weight ratio of 4.40 $\pm$ 0.42; Heart/Body weight ratio 2.43 $\pm$ 0.44; Lung/Heart ratio 1.79 $\pm$ 0.19, respectively. There were no significant differences of the ratios between sham surgery group and normal health group.

2) Effects of Aortic Obstruction on the Urinary Excretion of CA:
As Fig. 2 shows, there occurred significant increase in the urinary excretion of adrenaline and noradrenaline of 15 rabbits from 0.46 $\pm$ 0.14 mcg/day and 2.21 $\pm$ 0.54 mcg/day to 10.47 $\pm$ 3.72 mcg/day and 17.13 $\pm$ 7.34 mcg/day respectively in 24 hours after surgery, being followed by the prompt reduction, yet remained the higher level than that of the normal animals throughout the experimental period, showing 1.10 $\pm$ 0.40 mcg/day and 4.00 $\pm$ 0.35 mcg/day respectively even after 10 days. The five rabbits which underwent sham’s method revealed a transient increase of the urinary adrenaline and noradrenaline, up to 2.32 $\pm$ 0.61 mcg/day and 4.15 $\pm$ 1.13 mcg/day respectively in 24 hours, which are still markedly lower than the values found in

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Body weight (kg)</th>
<th>Heart weight (g)</th>
<th>Lung weight (g)</th>
<th>Lung/Body</th>
<th>Heart/Body</th>
<th>Lung/Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>15</td>
<td>2.45 $\pm$ 0.29</td>
<td>4.29 $\pm$ 0.35</td>
<td>7.43 $\pm$ 0.62</td>
<td>3.10 $\pm$ 0.19</td>
<td>1.80 $\pm$ 0.11</td>
</tr>
<tr>
<td>Sham oper.</td>
<td>5</td>
<td>2.54 $\pm$ 0.24</td>
<td>5.46 $\pm$ 0.42</td>
<td>9.59 $\pm$ 0.94</td>
<td>4.49 $\pm$ 0.42</td>
<td>2.43 $\pm$ 0.44</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>24</td>
<td>2.39 $\pm$ 0.45</td>
<td>7.43 $\pm$ 1.45</td>
<td>15.07 $\pm$ 4.62</td>
<td>6.29 $\pm$ 0.63</td>
<td>3.02 $\pm$ 0.21</td>
</tr>
</tbody>
</table>

Lung/Body weight ratio: lung weight (g)/body weight (g) $\times$ 1000
Heart/Body weight ratio: heart weight (g)/body weight (g) $\times$ 1000
Lung/Heart weight ratio: lung weight (g)/heart weight (g)

cardiac failure, the figures resumed the normal level eventually.

3) Effects of Experimental Cardiac Failure on Changes of CA Concentration of the Heart:

In 18 normal rabbits, average value of the CA of the whole heart was $1.50 \pm 0.24\, \text{mcg/g}$, the transient increase of $1.83 \pm 0.29\, \text{mcg/g}$ was observed in three hours after the blocking of the aorta; in a few days, however, it dropped sharply to $0.50 \pm 0.35\, \text{mcg/g}$, and continued to remain at the lower level in all instances throughout the period; while the heart CA of five sham animals showed $1.40 \pm 0.29\, \text{mcg/g}$ in two to ten days after the surgical procedure.

4) Effects of Aortic Obstruction on the Distribution of CA in the Various Organs:

As shown in Table III, the CA concentration in the normal rabbits showed to be higher in

---

Fig. 2. Changes of urinary excretion of catecholamine after aortic obstruction in rabbit.
left panel: Adrenaline excretion (mcg/day)
right panel: Noradrenaline excretion (mcg/day)
---: Cardiac Failure (15 cases)
.....: Sham operated (5 cases)
Vertical lines indicate standard deviations of the means

Fig. 3. Changes of catecholamine concentration of the whole rabbit heart after aortic obstruction.
Vertical lines indicate standard deviations of the means.

*Japanese Circulation Journal  Vol. 32, May 1968*
the heart, that is, the atria 2.04 ± 0.20 mcg/g; the left ventricle 1.54 ± 0.25 mcg/g and the right ventricle 1.45 ± 0.16 mcg/g. The figures of the other organs were, i.e., the spleen (0.74 ± 0.10 mcg/g); the aorta (0.61 ± 0.11 mcg/g) and the brain stem (0.51 ± 0.04 mcg), though the kidney, lung and liver disclosed the decreased value respectively. Adrenaline concentration in the adrenal gland showed overwhelmingly high value of 466 ± 166 mcg/g.

In the acute stage of cardiac failure, three hours after the obstructive procedure, there developed enhanced tissue CA concentration, i.e., the atria 2.27 ± 0.20 mcg/g; the left ventricle 1.84 ± 0.12 mcg/g; the right ventricle 1.59 ± 0.30 mcg/g; the spleen 0.83 ± 0.05 mcg/g and the adrenal gland 367 ± 38 mcg/g. During the subacute period, within 48 hours, however, reduction was noticeable and by the tenth day, the respective figures were as follows; the atria 0.98 ± 0.31 mcg/g; the left ventricle 0.80 ± 0.21 mcg/g; the right ventricle 0.34 ± 0.08 mcg/g; the spleen 0.34 ± 0.12 mcg/g and the adrenal gland 97 ± 43 mcg/g. Similar changes could be observed in the aorta, brain stem and liver, but with on appreciable changes in the

<table>
<thead>
<tr>
<th>Table III</th>
<th>Changes of Catecholamine Concentrations of the Rabbit Tissues before and after Aortic Obstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>cases</td>
<td>Normal 3 hours after oper.</td>
</tr>
<tr>
<td>Atria*</td>
<td>2.04 ± 0.20 (2.42–2.12)</td>
</tr>
<tr>
<td>Left ventricle*</td>
<td>1.54 ± 0.25 (2.02–1.66)</td>
</tr>
<tr>
<td>Right ventricle*</td>
<td>1.45 ± 0.16 (1.82–1.36)</td>
</tr>
<tr>
<td>Brain stem*</td>
<td>0.51 ± 0.04</td>
</tr>
<tr>
<td>Aorta*</td>
<td>0.61 ± 0.11</td>
</tr>
<tr>
<td>Lung*</td>
<td>0.18 ± 0.08</td>
</tr>
<tr>
<td>Kidney*</td>
<td>0.24 ± 0.08</td>
</tr>
<tr>
<td>Liver*</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>Spleen*</td>
<td>0.74 ± 0.10</td>
</tr>
<tr>
<td>Adrenal gland**</td>
<td>466 ± 166 (422–312)</td>
</tr>
</tbody>
</table>

Values are the means ± S.D.

*: calculated as noradrenaline (mcg/g)  **: calculated as adrenaline (mcg/g)

<table>
<thead>
<tr>
<th>Table IV</th>
<th>Uptake of Exogenous Noradrenaline by the Various Tissues in Rabbits with or without Cardiac Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal 4 cases</td>
</tr>
<tr>
<td>Heart</td>
<td>noninfused 7 cases</td>
</tr>
<tr>
<td>Atria</td>
<td>2.04 ± 0.20</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>1.54 ± 0.25</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>1.45 ± 0.16</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.74 ± 0.10</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>466 ± 166</td>
</tr>
</tbody>
</table>

Rabbits received noradrenaline (100 mcg/kg in 20 cc in Ringer's solution) intravenously for 30 minutes and were killed upon completion of infusion.

lung and kidney.

5) Uptake of Exogenous Noradrenaline by the Tissues:

To pursue the biochemical process in the trend of decreased tissue CA with simultaneous involvement of the increased urinary excretion in the experimental cardiac failure, the study on the tissue uptake of the endogenous CA has been attempted by intravenous infusion of the noradrenaline 100 mcg/kg in 20 cc in Ringer's solution for 30 minutes in the 4 normal and 4 animals with the heart failure, and they were sacrificed upon completion of parenteral administration of the drug. As Table IV, shows the noradrenaline concentration was definitely increased in the respective organ of normal animals, i.e., the atria from 2.04 ± 0.20 mcg/g to 3.86 ± 0.55 mcg/g; the left ventricle from 1.54 ± 0.25 mcg/g to 3.59 ± 0.19 mcg/g and the right ventricle from 1.45 ± 0.16 mcg/g to 3.98 ± 0.24 mcg/g. No noticeable increase could be seen in the rabbits of failing heart, i.e., the atria from 0.98 ± 0.31 mcg/g to 0.60 ± 0.39 mcg/g; the left ventricle from 0.80 ± 0.21 mcg/g to 1.14 ± 0.22 mcg/g and the right ventricle from 0.34 ± 0.08 mcg/g to 0.75 ± 0.21 mcg/g. While, adrenaline concentration of the adrenal gland had decreased in the normal similar to the failing animal in contrast to the increase shown in the other organs.

6) Effects of Nialamide, Propranolol and Bethanidine in CA Level of the Tissue and Urine:

To study the fate of CA in the sympathetic nerve endings in the condition of cardiac embarrassments, 29 rabbits with or without heart involvement over 10 days after obstructive intervention were intramuscularly administered of 10, 1 and 1 mg/kg of nialamide, the monoamine oxidase inhibitor, propranolol as the β receptor blocking agent and bethanidine which possesses the action of bretylium twice a day.

### Table V Effects of Nialamide, Propranolol and Bethanidine on Tissue Catecholamine Levels in Rabbits with or without Cardiac Failure

<table>
<thead>
<tr>
<th>Animal group</th>
<th>No.</th>
<th>Left * ventricle</th>
<th>Right * ventricle</th>
<th>Atria*</th>
<th>Spleen*</th>
<th>Adrenal** gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>1.54 ± 0.25</td>
<td>1.45 ± 0.16</td>
<td>2.04</td>
<td>0.74</td>
<td>466 ± 166</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>6</td>
<td>0.80 ± 0.21</td>
<td>0.34 ± 0.08</td>
<td>0.98</td>
<td>0.34</td>
<td>97 ± 43</td>
</tr>
<tr>
<td>Nialamide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>2.14</td>
<td>1.50</td>
<td>2.06</td>
<td>1.28</td>
<td>594</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>5</td>
<td>0.81 ± 0.19</td>
<td>0.48 ± 0.18</td>
<td>0.68</td>
<td>0.64</td>
<td>286 ± 69</td>
</tr>
<tr>
<td>Propranolol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>8</td>
<td>2.23 ± 0.19</td>
<td>2.03 ± 0.38</td>
<td>2.42</td>
<td>1.37</td>
<td>480 ± 134</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>7</td>
<td>1.12 ± 0.36</td>
<td>1.27 ± 0.53</td>
<td>0.81</td>
<td>0.77</td>
<td>304 ± 106</td>
</tr>
<tr>
<td>Bethanidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>2.84</td>
<td>1.62</td>
<td>1.43</td>
<td>0.97</td>
<td>570</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>3</td>
<td>1.35</td>
<td>1.08</td>
<td>0.89</td>
<td>0.80</td>
<td>400</td>
</tr>
</tbody>
</table>

*: calculated as noradrenalin (mcg/g ± S.D.)

**: calculated as adrenaline (mcg/g ± S.D.)

### Table VI Effects of Nialamide, Propranolol and Bethanidine on Urinary Excretion of Catecholamine in Rabbits with or without Cardiac Failure

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Adrenaline (mcg/day)</th>
<th>Noradrenalin (mcg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Cardiac failure</td>
</tr>
<tr>
<td>Non treated</td>
<td>0.46 ± 0.14</td>
<td>0.77 ± 0.33</td>
</tr>
<tr>
<td>Nialamide</td>
<td>0.36 ± 0.20</td>
<td>0.79 ± 0.23</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.69 ± 0.34</td>
<td>0.89 ± 0.28</td>
</tr>
<tr>
<td>Bethanidine</td>
<td>0.33 ± 0.10</td>
<td>0.36 ± 0.09</td>
</tr>
</tbody>
</table>

*Japanese Circulation Journal Vol. 32, May 1968*
for five days respectively. Urinary excretion of CA before and during the administration of these agents and tissue concentrations were estimated. The Table V, VI indicate the fact that the CA concentration of the left and right ventricles, spleen and adrenal gland had elevated while the urinary CA of adrenaline and noradrenaline tended to decrease with nialamide and bethanidine in the normal animals. With propranolol, the tissue CA showed an increase along with enhanced adrenaline excretion, yet with unchanged noradrenaline content. In the failing heart, however, the tissue CA exhibited suppressed effect.

7) Effect of Reserpine on the Failing Heart of the Rabbit:

To study the pathophysiological aspect of CA accompanied of the failing heart 12 rabbits were administered intramuscularly 2 times a day with 0.1mg/kg of reserpine. As Fig. 4 indicates, the levels of urinary CA and of the whole heart showed striking decrease with administration of reserpine. The urinary adrenaline decreased from 0.46±0.14 mcg/day of nontreated rabbit to 0.30±0.09 mcg/day, while that of noradrenaline from 2.21±0.54 mcg/day to 0.54±0.33 mcg/day, and CA of the heart dropped from 1.50±0.24 mcg/g. to 0.25 mcg/g. Two out of 10 rabbits treated with reserpine developed of fatal pulmonary edema following aortic obstruction. In the survived animals, the pulmonary congestion and edema became more pronounced than that of the nontreated animals with cardiac failure. (Table VII)

![Diagram showing changes of heart and urinary catecholamine levels after aortic obstruction in reserpinized rabbits. Each rabbit was given reserpine (0.1mg/kg twice a day i.m.) till the sacrifice.]


<table>
<thead>
<tr>
<th></th>
<th>Normal (15)</th>
<th>Sham operated (5)</th>
<th>Cardiac failure (24)</th>
<th>Reserpinized and operated (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung/Body</td>
<td>3.10±0.19</td>
<td>4.40±0.42</td>
<td>6.29±0.63</td>
<td>7.10±0.84</td>
</tr>
<tr>
<td>Heart/Body</td>
<td>1.80±0.11</td>
<td>2.43±0.44</td>
<td>3.02±0.21</td>
<td>2.86±0.37</td>
</tr>
<tr>
<td>Lung/Heart</td>
<td>1.70±0.24</td>
<td>1.79±0.19</td>
<td>2.27±0.43</td>
<td>2.60±0.39</td>
</tr>
</tbody>
</table>


discussion

Much has been reported on the production of cardiac failure by experimental method. In this study a method tried by UAEA-Oda has been followed using the rabbits. The procedure requires no open chest surgery and with minimum operative procedure. With the sham operation which necessitates only the vinyl tube insertion, variation of urinary and heart CA revealed considerably lower figures. This technique is simple and dependable, out of 78 operative cases, only 6 failures were encountered due to slipping of the laminallia from the vinyl tube.

In 1955, the fluorometric analytical method for measuring of CA was introduced, yet reports on CA distribution in the organ in animals have been limited. It has been known that the tissue CA was found to be rich in the adrenal gland, heart and spleen. In the present study, the CA concentration of the various organs of the rabbits was measured and found that the heart contained the greatest amounts being followed by the spleen. Its concentration is reduced in the order of aortic wall, brain stem, kidney, liver and lung. Our findings were essentially identical with those of Shore and Carlsson despite their variable methods of extraction and assay. Assumption was made that CA concentration of the tissues would closely parallel with the degree of heightened activity of the sympathetic nerves, hence, the variation of CA in the heart would likely be followed the functional mode of the autonomic nervous system. The higher CA content has been found in the atria than ventricles and higher in the right side of the heart than the left side, particularly noticeable in the sinus node with its stimulation conduction system. It is hardly possible for us to explain the myocardial distribution of CA solely from the aspect of the sympathetic function, since CA concentration in the sinus node is not necessarily high according to Shore et al. and the CA is detectable even after denervation. It was shown that the highest concentration of noradrenaline was found in the atria (2.04±0.20 mcg/g), followed by the left ventricle (1.54±0.25 mcg/g) and the right ventricle (1.45±0.16 mcg/g). The noradrenaline concentration of the right ventricle was less than that of left ventricle, perhaps the method of myocardial sectioning different from other reporter may have responsible for such variable figure. The adrenaline concentration of tissue was overwhelmingly higher in the adrenal gland (466±106 mcg/g), compared with the other organs which contained lesser, and in the heart adrenaline concentration was found to be less than 3 per cent of noradrenaline value. In this report, the CA of the adrenal gland was based on adrenaline content while noradrenaline estimation was used in other organs, mainly because of certain technique difficulty.

When obstructive aorta was established, the CA concentration of the heart (mean value 1.50±0.24 mcg/g) rose to 1.83±0.29 mcg/g, thereafter began to fall quickly and reached about one third of the normal value in three days. The urinary excretion of CA, on the contrary, showed distinct increase during the postoperative period followed by fall but above the normal range. While a number of papers on the human urinary excretion of CA of the cardiac failure have reported but none of them were conclusive. Raab has stated that he found no significant changes to be observed. Pekkanen had detected some increase in the urinary adrenaline but noradrenaline in some patients with cardiac failure of moderate severity and stated that increase would be much less in the advances cases which were not amenable to treatment. Matsumoto observed that the

*Japanese Circulation Journal Vol. 32, May 1968*
Tissue Catecholamine Concentration of Rabbit with Cardiac Failure

Trend of increasing pattern of urinary CA corresponding to the peak of the III grade of cardiac failure based on the classification of New York Heart Association, while Chidsey has observed elevation only in the patient with severe cardiac failure as grade III or IV.

Stimulation of the sympathetic nerves to the adrenergically innervated tissues causes remarkable increase of CA content in the venous effluent. There appears to be positive correlation between the venous CA concentration and urinary excretion of CA which was measurable with urine collected for about one hour during which a specimen of blood withdrawn for determination. When the exogenous CA was administered intramuscularly or intravenously to the animal or man, two to five per cent would be non-metabolized and excreted in urine, irrespective of the presence of the liver and renal disturbances. Therefore, the increased CA excretion seems to reflect the rise of CA level in blood, which reasonably suggested that cardiac failure yields the accelerated sympathoadrenal activity.

The detectable increase was found in two out of four rabbits in the initial obstruction period, but the tissue CA was found to be reduced markedly in a few days after the circulatory intervention. Chidsey and Spann reported pronounced diminution of the myocardial concentration of CA in the dog and guinea pig with experimental cardiac failure, with the emphasis that reduction would bound to occur in the ventricle receiving direct cardiac load which could not be observed in the other organs such as the kidney. It is unlikely that the changes of tissue CA level should be limited only to the heart, however, study on the tissue CA showed diminution not only in the heart, but also in the spleen, adrenal gland and others.

In general, the CA is conceded to be synthesized locally in sympathetic nerve endings or chromaffin cells, partially derived from the circulation and stored in the vesicles of the nerve endings. With the use of $^3$H labelled noradrenaline, Kopin found that the rat heart took up as much as 20 per cent of noradrenaline content of the heart from the circulation. It has been believed, these stored CA in the tissue is present in at least two different metabolic pools.

One is a type of active CA with a rapid turnover, rate released easily from the nerves by sympathetic nerve impulse or tyramine, and the other is a type of bound CA which turns over slowly and is relatively refractory to release by tyramine, reserpine or sympathetic nerve stimulation.

In order to make further confirmation of the mechanism that the marked reduction of tissue CA is to be found in cardiac failure, it is necessary to refer the mechanism of the uptake, synthesis, store and release of CA at the sympathethic nerve ending. When 100 mcg/kg of noradrenaline was administered intravenously to the rabbit with or without cardiac failure, the CA concentration had markedly increased in the normal rabbits, i.e., in the left ventricle 3.59 mcg/g, the right ventricle 3.98 mcg/g and the atra 3.86 mcg/g respectively but these increases could be significantly suppressed in cardiac failure, that is, the left ventricle 1.14 mcg/g, the right ventricle 0.75 mcg/g and the atra 0.60 mcg/g respectively. Oliverto was able to demonstrate uptake of the rabbit heart by using $^3$H labelled noradrenaline and found 30 per cent lower in cardiac failure than that in normal animal. Even though only about 20 per cent of noradrenaline content of the heart would be derived from the blood stream, it is hardly possible to explain the lessened myocardial CA of rabbit fallen to one third of the normal value only by abnormal uptake. Increased sympathoadrenal tension by the sympathetic stimulation leads to accelerated noradrenaline synthesis, so that CA reduction is either none or negligible despite heightened CA release. It should be considered that the failing heart may enhanced the noradrenaline synthesis.

The transient increase of the heart CA observed in the study, from 1.50±0.24 mcg/g to 1.83±0.29 mcg/g, could be interpreted as the synthetic process of CA and uptake tended to exceed the rate of release and metabolic rate at the nerve ending. The active noradrenaline is liberated from the sympathetic nerve endings in accordance with impulses received under the hyperactivity of the sympathetic nerves. A part of it interacts with nearby receptor to exert physiologic effect, while the remainder is rebound with the nerve ending or possibly

*Japanese Circulation Journal Vol. 32, May 1968*
washed out from its site of action in the blood stream\textsuperscript{34} or otherwise inactivated by catechol-O-methyl transferase (COMT). On the other hand, the bound noradrenaline replaces the loss of active noradrenaline and excess had to be transformed by monoamine oxidase (MAO), which enabled regulatory maintenance of CA storage. Although administration of nialamide, bethanidine and propranolol which tends to inhibit the metabolic process, mobilization and receptor action of CA, yet, concentration of the heart, spleen and adrenal gland were inducible to increased CA with or without cardiac failure. However, the increase of tissue CA could be suppressed significantly in cardiac failure even with the administration of nialamide or bethanidine which has tendency to enhance bound noradrenaline, particularly by nialamide no changes have been observed. With these drugs CA content of urine would be decreased though no distinction could be seen between the normal heart and failing one. It has been suggested that in cardiac failure, variation of CA by nialamide would be responsible to disturbed CA metabolism including increased MAO activity and that by bethanidine to enhanced release of CA exceeding blocking effects of mobilization in the sympathetic nerve ending.

It is most probable that diminished CA concentration in the various organs of the failing heart implies a concept that noradrenaline is released actively in the receptor body in response to hyperactivity of the sympathetic system, exceeding the synthesis and uptake of noradrenaline which lowered stored bound form. Therefore it is suggested that in the failing heart, there is an increased turnover rate of tissue CA, especially in the myocardium. According to Spann\textsuperscript{29}, no significant difference could be observed in the turnover rate of CA in the guinea pig with or without cardiac failure as far as \textsuperscript{3}H noradrenaline is concerned. With propranolol, the tissue CA is significantly increased with high urinary adrenaline, though with unchanging content of noradrenaline. Extent of these changes is definitely lowered when compared with normal. The increased excretion of adrenaline by propranolol is supposedly due to hyperfunction of the sympathetic nervous system by homeostatic mechanism in response to the blockade of stimulating effect of CA at the $\beta$ receptor. Euler\textsuperscript{48} has reported that propranolol inhibited both the uptake and release of noradrenaline in the sympathetic nerve ending. From above observation, a deduction could be reached that the increase of tissue CA by propranolol could be caused by enhanced synthesis and altered metabolism.

The role of endogenous CA of myocardium in maintaining the cardiac function has not been established. Lee\textsuperscript{40} was able to demonstrate an important physiological participation of the myocardial noradrenaline on maintaining muscular contractility by the experimental depletion of CA by denervation or use of reserpine in cat. A number of similar results have been reported\textsuperscript{41,42}. On the other hand, some investigators have held the opinion that experimentally induced noradrenaline depletion in animals does not necessarily signify the severe impairment of myocardial contractility\textsuperscript{43,44,45}. Matsumoto\textsuperscript{46} made clinical observation that reserpine would aggravate the symptoms of the patients with cardiac failure. Gaffney\textsuperscript{47} also reported such worsening of clinical manifestation with guanethidine. These condition have proved the evidence that drastic depletion of myocardial CA would lead to marked pulmonary edema which made higher mortality when aortic obstruction was produced; undoubtedly, the cardiac CA is necessary for regulation of the myocardial function. But the direct action of reserpine\textsuperscript{42} to myocardium should be kept in mind when faced with CA depletion in the organs.

**Summary**

The pathophysiological significance of CA in cardiac failure was studied by determining the urinary excretion of CA, their concentration in the various tissues of the rabbit experimentally developed of cardiac failure by artificial causation of the aortic obstruction.

1) Urinary excretion of CA, namely, adrenaline and noradrenaline, were increased significantly in a few days after surgery being followed by gradual reduction, but maintained the higher level over the normal range throughout the experimental period.

2) CA concentration of the whole heart aver-
aged 1.50±0.24 mcg/g in the normal rabbit had increased up to 1.83±0.29 mcg/g in three hours after the operative procedure and followed by the prompt reduction down to about one third of the normal value and maintained the lower level as compared to normal range.

3) The uptake of exogenously infused noradrenaline by the heart was found to be suppressed significantly in the rabbit with cardiac failure as compared to that of the normal animal.

4) The intramuscular administration of nialamide, propranolol and bethanidine have produced the following results.

(A) Nialamide and bethanidine administered in the normal rabbits produced the increase of the CA concentration of tissues, especially the left ventricle, while the urinary excretion of CA had decreased, both adrenaline and noradrenaline. In cardiac failure, the tissue CA increase was lower than that in the normal, particularly with bethanidine.

(B) Propranolol produced the increase of the CA concentration of the heart, spleen and the urinary adrenaline, but no change in the urinary noradrenaline. With propranolol, changes of tissue CA were suppressed in cardiac failure.

It has been shown that practically no changes of myocardial CA could be observed in cardiac failure by nialamide. That the presence of another metabolic pathway of the CA except MAO in cardiac failure has been suggested.

5) Deficiency of the heart CA incident to the experimental aortic obstruction in rabbit develop pronounced pulmonary edema causing a rise of mortality.

It is concluded that the failing heart yields the acceleration of the sympathoadrenal activity and results in a reduction of the tissue CA, especially in the myocardium. It is assumed that the reduced tissue CA in cardiac failure might be due to the increased release of the stored CA, or the deficiency of its retention in the sympathetic nerve ending which responsible to a rise of turnover rate of CA, or a lowered uptake. The myocardial CA may play an important compensatory role for the regulation of the failing cardiac function.

Acknowledgement

The author expresses his sincere gratitude to Professor Tatsuya Tomomatsu for his kind guidance, and to Dr. Y. Ueba, Dr. M. Kondo, Dr. M. Oda, Dr. Y. Ijiri, Dr. H. Kogame and Dr. T. Yao for their cooperation.

REFERENCES

26. Iyiri, Y.: to be published.