A New Experimental Device for the Measurement of Moisture Emission and Heat Release from Respiratory Organs and Body Surface

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A new experimental device was developed to investigate respiratory diseases. The moisture and heat released from respiratory organs and the body surface of a rat were determined by means of this device as well as the rectal temperature. The high recovery of results was statistically confirmed, and the measured values at various environmental temperatures were significantly different from each other. Some standard drugs, such as ephedrine, aminophylline and chlorpromazine, were examined. Their stimulant or depressant actions were clearly observed. The results of some traditional medicines for the treatment of rhinitis and bronchial asthma from this measuring system were consistent with their clinical applications. These results suggest that this new experimental system is not only effective in the experimental understanding of cold-hot syndrome, but also contributes to the evaluation of the effects of traditional medicines.

Key words rhinitis; bronchial asthma; body temperature; water metabolism; traditional medicine

During the second half of the 20th century, advances in science brought about the rapid development of medical treatment embracing diagnostic and surgical technology and chemical drugs. As a result, great changes have taken place in the types of disease prevalent, and mortality has declined by a large degree in advanced countries. However, modern civilization has exposed mankind to complications of social life, various stresses and many changes in life habits at the same time. A lot of people are suffering from homeostasis disorder and are complaining of being in a slump because these irritations have surpassed the margin of their adaptability. Also, multi-organ disorders are increasing due to the lowering of homeostasis with aging. All of these factors have decreased the quality of life and have become important reasons for chronic disorders. As obstacles to people's health, they are among the important subjects of modern medical treatment and are turning into social problems.

That is to say that nowadays, important diseases are concerned with homeostasis disorder, which is the balance of function in the final analysis. As yet, modern science cannot understand the essence of homeostasis. Thus, it has long way to go to scientifically understand life function and interpret these diseases.

On the other hand, the ancient Chinese divided all phenomena in the world into two categories prior to the emergence of natural science, namely, "Yin (陰)" and "Yang (陽)." Their interactions mean mutual control, mutual masking and mutual balance. Sharing these principles, traditional Chinese medicine applied this "Yin-Yang Theory" to explain life function and its disorders, and established its corresponding therapeutic system. Because such a theory aims primarily at understanding homeostasis disorder, its significance has generally been acknowledged. Moreover, many clinical achievements of traditional medicines have been reported. Consequently, scientific understanding of traditional Chinese medicine has become an important subject for their effective and flexible application in clinical practice.

In traditional medical theories, "cold (寒)" and "hot (熱)" are a pair of the most basic and important elements. They don't simply imply the levels of body temperature or regional temperature of body. Cold-hot syndrome (寒熱症) not only includes physiological conditions of the body with changes in body temperature, but also involves the distribution and balance of body fluids with these changes. Especially, respiratory diseases such as rhinitis and bronchial asthma, as well as neuralgia or arthralgian of the extremities are also closely related to water metabolism and its distribution in the corresponding region. Thus, we thought that the cold-hot syndrome of the respiratory system may be understood by the measurement of moisture and heat released from respiratory organs and body surface, considered together with body temperature.

Based on the points mentioned above, we developed a new device that is characterized by the simultaneous measurement of moisture emission and heat release both from the body surface of a rat and with its panting, as well as the rectal temperature. This report encompasses the recovery, on the experimental device, of the effects of some standard drugs and traditional medicines on moisture emission and heat release from respiratory organs and body surface as well as body temperature. Our primary objective is to define characteristic effects of traditional medicines used to treat rhinitis and bronchial asthma by using this device, and to provide direct evidence for the clinical use of these medicines.

MATERIALS AND METHODS

Drugs Chlorpromazine (Wako Pure Chemical Industries, Ltd., Japan), ephedrine hydrochloride (Dainippon Pharmaceutical Co., Ltd., Japan) and aminophylline (Sanseki Pharmaceutical Co., Ltd., Japan) were dissolved in distilled water before the experiments, respectively. Materials of traditional medicines were Sho-seiryu-to(SST), Ryokan-kyo-mingen-to(RKS) and Makyo-kanski-to(MKK) provided by Kanabo Pharmaceutical Co., Ltd., Kotaro Kampo Pharmaceutical Co., Ltd. and Asahi Beer Pharmaceutical Co., Ltd., respectively. Their details are listed in Table 1. Each material was dissolved and/or suspended in distilled water and kept at 25°C before the administration.

Animals Male Wistar rats (Nihon SLC Co., Ltd., Japan), 4 weeks old, were housed in groups of 2 or 3 per cage with free access to water and food. Housing was air-conditioned at

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Table 1. Details of Traditional Medicines

<table>
<thead>
<tr>
<th>Materials</th>
<th>Daily dose (mg)</th>
<th>Experimental dose (mg/kg)</th>
<th>Content of main components (mg/daily dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SST</td>
<td>5200</td>
<td>1148</td>
<td>ephedrine: 17.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>glycyrrhizin: 30.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>glycyrrhizin: 23.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>amygdalin: 136.8</td>
</tr>
<tr>
<td>RKS</td>
<td>4500</td>
<td>1350</td>
<td>ephedrine: 35.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>glycyrrhizin: 60.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>amygdalin: 102.4</td>
</tr>
<tr>
<td>MKK</td>
<td>2250</td>
<td>625</td>
<td></td>
</tr>
</tbody>
</table>

Quantitative analysis of the main components was carried out on a dual Shimadzu LC-6AD system equipped with a Shimadzu SPD-10A detector and its data processor: Conditions were as follows: column, Inertsil ODS-2 (4.6×250 mm, GL Science Inc.); column temperature, 40°C; flow rate, 1.0 ml/min; wavelength: ephedrine: 210 nm; glycyrrhizin: 248 nm; amygdalin: 214 nm; mobile phases, ephedrine: 0.005 n SDS, 0.005 n Na,HPO₄ (adjusted to pH 3.0 with H₂PO₄), MeCN (61:39); glycyrrhizin: 0.01 n KH₂PO₄ (adjusted to pH 2.2 with H₂PO₄), MeCN (64:36); amygdalin: H₂O-MeOH (85:15); injection volume, 10 μl. The content of each component was calculated according to the absolute calibration curve method.

the temperature of 23±1°C, with a R.H. of 50±2% and a 12-h light-dark cycle. Rats, from 4 weeks old, underwent certain training that made them adaptable to the device. The training didn’t stop until the rat in the measuring conditions gave stable charts of temperature and humidity, a process which took about 2 weeks. Well-tamed rats weighing 190—230 g were used for the experiments. They were fasted for about 18 h before the experiments.

Measuring System This new device was made of a box that was divided into two rooms (head room and body room) with a pillory. When a rat with the pillory was boxed in it, its head and body were set in the two separate rooms so that moisture and heat released from the expired air and body surface could be measured separately. There were two pipes, one inlet and another outlet, attached to each room. The temperature, humidity and flow rate of the air flowing into the device were kept constant under thermal- and humidity-controlled chamber and two flowmeters, respectively. The humidity and temperature of the air leaving each room were measured in each outlet with a converter, respectively (Fig. 1).

The instruments used in the experiments were as follows: constant temperature and constant humidity chamber (EYELA, KCL-1000, Tokyo Rikakikai Co., Ltd., Japan), temperature and humidity converters (YH-22, Semato Engineering Co., Ltd., Japan), recorder (TR-250, Tokyo Rikakikai Co., Ltd., Japan), air pump (CHKARA, a-400SW, Nisso), flowmeter (KOFLOC, RK-200, Kojima Instruments Co., Ltd., Japan), digital thermometer (OMRON, MC-148, Matsuzaka Tateishi Denki Co., Ltd., Japan).

Experimental Procedure The experiments were performed between 12:00 and 18:00. First, a rat was treated with normal saline and the trial was done in 30 min. After 30 min of measurement, the rat was taken out of the device and its rectal temperature was examined at once. Subsequently, a trial in which the rat was treated with drugs started in 2 h. Each procedure was the same as that of the control trial. Only the rats that gave normal charts were used for drug trials.

Assay of Moisture Emission and Heat Release The humidity and temperature of the air leaving the head room and body room were recorded simultaneously and respectively by recorders connected to temperature-humidity converters.

They were analyzed according to the charts. The relationships between humidity and its peak height at different temperatures (32°C, 22°C, 11°C), as well as those between temperature and its peak height at R.H. of 40 or 45% were inspected. With good linearity, corresponding regressive equations were obtained. The humidity and temperature were calculated according to their peak height and the regressive equations.

On the basis of the humidity during the 30 min of measurement, the moisture emission (μl/h) from expired air or body surface in 1 h was calculated using the following formula:

\[ (\mu l/h) = \frac{V.P. \times \text{moisture} \times \text{MW} \times \text{F.R.} \times \text{of air} \times \Delta H}{V.P. \times \text{water at 100°C} \times \text{molar volume of H₂O}} \]

where V.P. is the vapor pressure in mmHg, MW the molecular weight in mg, ΔH the incremental changes in moisture humidity in %, F.R. the flow rate in l/h, and the molar volume in ml.

Released heat (°C) was expressed as the air temperature at the outlet minus that at the inlet.

Statistical Analysis All values were expressed as the means±S.D. Student’s t-test for unpaired or paired observations was used for the statistical evaluation of recovery on the measuring system or of the effects of standard drugs and traditional medicines, with a p value of 0.05 less considered significantly different.
RESULTS

Recovery on the Measuring System  As shown in Fig. 2, when rats were orally given 1ml of normal saline, it was statistically confirmed that the measured values in the normal, higher or lower temperature environments were both of high recovery and of significant difference from each other, all of the p value being less than 0.001 in comparison with the normal condition. Further, it was interesting to note that the moisture emission from expiration or the body surface of the rats at the environmental temperature of 32°C was approximately doubled compared with those at 22°C and the rectal temperature rose nearly 2°C. Also, at 11°C, the moisture emissions were about 50% those at 22°C, and the rectal temperature fell by about 1°C.

Effects of Some Standard Drugs by the New Measuring System  Ephedrine given orally at 7.37 mg/kg produced a rise in body temperature of 0.2°C to 1.8°C against the corresponding controls. At various temperatures, it resulted in a remarkable increase in moisture emission from expired air. However, it depressed or tended to depress heat release from the body surface in normal or cold environments (Fig. 3).

Aminophylline given orally at the dose of 20 mg/kg raised body temperature 0.8°C to 1.6°C. Moreover, marked increases in moisture emission and heat release from both expiration and the body surface were confirmed (Fig. 4).
The effects of chlorpromazine, which was given intraperitoneally at 2.0 mg/kg, are shown in Fig. 5. It was observed that chlorpromazine, contrary to ephedrine or aminophylline, produced a decrease in body temperature of 0.7°C to 1°C. In a higher temperature environment, it also depressed the moisture emission from both expired air and the body surface, both being decreased by about 30%. However, depression of the heat release from the body surface was definite only at an environmental temperature of 32°C.

**Effects of Some Traditional Medicines by the New Measuring System**

SST was given orally at 1148 mg/kg, in which the ephedrine content was the same as that of standard ephedrine. It was found that SST produced an increasing effect on the moisture emission from expired air, similarly to that of standard ephedrine, and the body temperature increased 0.4°C to 2.0°C above the corresponding controls. In addition, significant increases in heat release from both expired air and the body surface were clearly seen (Fig. 6).

The effects of RKS given orally at 1350 mg/kg are shown in Fig. 7. RKS resulted in an increase in body temperature of 0.3°C to 0.5°C, which seemed to be more remarkable in the low temperature environment. Moreover, slight increases in moisture and heat released from the body surface were observed.

MKK was given orally at 625 mg/kg, in which the ephedrine content corresponded to 9.82 mg. In body temperature, there was an increase of about 0.8°C at the environmental temperature of 32°C, whereas there was little increase under the normal or cool conditions, as compared with corresponding controls. Also, MKK increased only the moisture emission from expired air, whereas there were no clear changes in that from the body surface. At the same time, some decreases in heat release from either expired air or the body surface were confirmed (Fig. 8).
DISCUSSION

In homothermic animals, body temperature is generated mainly by the energy arising from metabolism and movement and is regulated by heat loss by way of skin, expiration and so on. As man reaches a hot or fevered state due to movement, environmental factors or illness, his thermo-regulatory centers are activated to control body temperature mainly by sweating because human has developed an eccrine sweat gland. However, under a normal environment, sweating by the eccrine sweat gland does not come into operation for active regulation of body temperature. Especially, if heat loss from the body surface is controlled by clothing, etc., in a cold environment, it means that man’s body temperature is influenced largely by expiration, similarly to such small animals as birds or rats.

The heat loss of the body depends closely on the amount of vapor in the lungs and on the water volume of insensible transpiration. Thus, in addition to urination, the regulation of body fluids also involves insensible transpiration and expiration, and these processes are related to body temperature. Clothing can control transpiration, but breathing can’t cease. By breathing, perpetual heat release and perpetual water emission are performed.

In traditional medicine, the lungs are the organs in charge of oxygen intake which is the source of Ki(死者) energy and the regulation of body fluids. Clinical evidence suggests that traditional medicines for the treatment of rhinitis and asthma cannot inhibit the formation of IgE antibody, but are concerned with another mechanism which involves the inhibition of Lichtenstein’s histamine release at stage 1.10,11 It is believed that respiratory diseases such as rhinitis and bronchial asthma have a close correlation with the maintenance of body fluids and the local temperature of the respiratory system. Similarly, external humidity accompanied by transpiration also has a great influence on neuralgia or arthralgia of the extremities. There are inseparable relationships between some diseases of the respiratory system or the extremities and the Cold-hot syndrome, together with water metabolism, which is one of the basic theories of traditional medicine.

In an attempt to elucidate the pharmacological properties of traditional medicines, we developed a new device to experimentally investigate the disease condition caused by disturbances of the regulation of body temperature, i.e. Cold-hot syndrome. This device is characterized by the simultaneous measurement of moisture and heat released from both expired air and the body surface, as well as measurement of body temperature.

The basic data for the device and its recovery are shown in Fig 2. Through experiments on 30 to 50 rats in groups of 7 to 10 rats for each measuring condition, it was statistically confirmed that all of the measured values were both of low standard deviation and of significant difference from each other. At temperatures of 32°C or 11°C and a humidity of 40% or 45%, the moisture emission from expired air or the body surface increased or decreased, as compared to those under the normal condition (22°C, 40%). Similar results were observed in terms of rectal temperature. The heat release, however, was more remarkable in the lower temperature environment. Because of its good recovery, this measuring system seems to be highly reliable.

Subsequently, the effects of some standard drugs were examined by means of it. Ephedrine is not only widely used for asthma but is also a constituent of many remedies. It produced a marked rise in body temperature and a marked increase in moisture emission only from expired air. However, there were no clear changes in heat release. Aminophylline, as a bronchodilator, showed similar effects on body temperature and moisture emission from expired air to those of ephedrine. At the same time, it also led to a potent increase in moisture emission from the body surface as well as heat release from both expired air and the body surface. Although both are antiasthmatics, there are clear differences in the route of water emission or heat release between them, which were revealed by the new device. For the purpose of comparison with ephedrine and aminophylline, chlorpromazine, which is a major tranquilizer which acts to lower the body temperature, was examined. It depressed the water emission from respiratory organs with the decrease in body temperature. Moreover, some depression of water emission from the body surface or heat release was confirmed.

SST, RKS or MKK, with or without ephedra herb or ephedrine, are all traditional medicines used to treat bronchial asthma, but they have different clinical applications. SST used in the study contained the same amount of ephedrine as that of standard ephedrine. Although it maintained the increasing actions of ephedrine on body temperature and moisture emission from expiration, it also tended to enhance the evaporation of water from the body surface. And the marked increases in the heat release from expired air and body surface were shown, in which SST considerably differed from ephedrine. It was observed that SST showed more powerful effects on the improving in the storage of body flu-
ids in respiratory organs than those of standard ephedrine. Clinically, rhinitis with watery secretions and bronchial asthma with water-like sputa are attributed to the decrease in moisture emission from respiratory organs, which deals with the depression of vapor pressure of the expired air caused by a lowered regional temperature of the respiratory organs. The results from our study are consistent with the clinical uses of SST for rhinitis with watery secretions or bronchial asthma with water-like sputa. They may also account for the clinical report\(^2\) that the administration of SST aggravated atopic dermatitis, accompanied by strong itching.

It is said that RKS was developed from SST, even though it contained no ephedra herb.\(^2\) RKS is used with disease conditions with more storage of body fluids than that of SST, \textit{i.e.} with the tendency towards edema. According to the results, its actions were more similar to those of aminophylline than to those of ephedrine, although these actions were mild or weak. Thus, if RKS clinically resulted in the disappearance of edema, this action should be owed to urination rather than to moisture emission from respiratory organs or skin. The above results also suggest that the actions of RKS on the improvement in symptoms of respiratory organs may be secondary actions from improvement of the whole body condition. This is good evidence for the clinical report\(^5\) that patients with thirst, heat, and sticky sputa became worse following the administration of RKS.

The characteristic effects of MKK were confirmed, which are greatly different from those of SST or RKS. Even though the ephedrine content in MKK used in our study was greater than that in SST, there was no clear rise in body temperature. Meanwhile, the heat release from both expired air and the body surface showed a tendency to decrease, whereas the moisture in expired air was markedly increased. Such results explained the clinical uses of MKK corresponding to children's dry cough with heat in the lungs. MKK, used for bronchial asthma with fever, thirst, and sticky sputa, is effective in relieving an asthmatic attack by means of the relaxant action of ephedrine on bronchial smooth muscle (\(\beta_2\)-action) without raising body temperature. Moreover, the increase in moisture emission from the lungs contributed to the improvement in the drying conditions of the airway, which is useful data for the clinical uses of MKK. The fact that MKK helps maintain the bronchodilation caused by ephedrine and does not raise body temperature should be attributed to the effects of gypsum, which is a cleaning and cooling agent for fever according to traditional medicine. There has been little experimental evidence for such an effect of gypsum until now. It is of further interest that its antagonistic effect against increase in body temperature caused by ephedrine was elucidated by the new device.

As a system to measure regional body temperature and tissue moisture, its ability to reveal certain aspects of drug effects suggests that it may be useful in the elucidating traditional medicines.

Studies of the effects of traditional medicines on variations in the diurnal rhythm of mouse were reported.\(^13\) Using a machine\(^14\) for the measurement of heat release has the advantage of long-term measuring without restraint. However, that machine measured only the heat released from the whole body, without separating the expired air and that released from the body surface. As the machine did not correct the gasification correspondent to vapor from expiration and transpiration, and failed to control the balance between the two related moisture amounts, the true heat was not measured. However, management of these aspects are characteristic of our measuring system.

At present, we are investigating the difference between animals in a higher temperature-lower humidity condition and those in a lower temperature-higher humidity condition, as well as variations due to racial difference and the responses of sensitized animals after the administration of traditional medicines. Through these studies, we made an attempt to understand not only the pharmacological actions but also the physiological actions of drugs by the new measuring system. It may provide helpful information about the pharmacological properties of traditional medicines.

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1) Takagi K., Yamamura H., “Toyoigaku wo Manabuhito No Tenmai (東洋医学を学ぶ人のために),” Igakusyoin, Tokyo, 1984, pp. 261—266.
8) a, Sho-seiryu-to (四神膏); prescription: [Ephedra Herb (麻黄) 3.0 g, Pinellia Tuber (半夏) 5.0 g, Licorice (甘草) 5.0 g, Peony Root (芍薬) 3.0 g, Dry Ginger (乾薑) 3.0 g, Cinnamon Bark (桂皮) 3.0 g, Asiasarum Root (細辛) 3.0 g, Schisandra Fruit (五味子) 3.0 g]. Extract powder of SST was mixed with 70% methanol and extracted with a ultrasonicator for 40 min. The mixture was centrifuged at 3000 rpm for 20 min. and the supernatant solution was evaporated in vacuo. 1 g of extract powder yielded 0.53 g of powder of condensed extract. b, Ryokan-kyouni-shingenin-to (苓甘姜辛煎); prescription: [Porzi Sclerotium (茯苓) 4.0 g, Licorice (甘草) 2.0 g, Schisandra Fruit (五味子) 3.0 g, Dry Ginger (乾薑) 2.0 g, Asiasarum Root (細辛) 2.0 g, Pinellia Tuber (半夏) 4.0 g, Apricot Kernel (杏仁) 4.0 g, c, Makoyakanseki-to (麻杏甘石湯); prescription: [Ephedra Herb (麻黃) 4.0 g, Apricot Kernel (杏仁) 4.0 g, Licorice (甘草) 2.0 g, Gypsum (石膏) 10.0 g].
14) BioDynamic Calorimeter BDC-200 (ESCO, Tokyo).