# CHAPTER II

# MATERIALS AND METHODS

## Myometrial Specimens

The myometrial specimens used in this study were obtained from 54 women, aged 44.13 ± 5.41 (32 to 52) years old, who underwent hysterectomy because of benign diseases under general (n = 12) or spinal anesthesia (n = 42) at the Department of Obstetrics and Gynecology, Chulalongkorn Hospital. The indications for hysterectomy were myoma uteri (n = 38), recurrent dysfunctional uterine bleeding (n = 8), adenomyosis (n = 5), left ovarian cyst (n = 1), and prolapsed uterus (n = 2). All myometrial specimens were obtained from women during proliferative phase of the menstrual cycle. The muscle strips were taken from fundus of the anterior wall of the uterus. The specimens were proved normal by gross and microscopic examination, and were also studied to verify the proliferative status of endometrium.

## Preparation of Myometrial Tissues

The muscle strips were immediately immersed in cold Tyrode's solution. The specimens were taken to the laboratory and then bubbled with a gas mixture of 95 % oxygen and 5 % carbon dioxide.

The circular layer of myometrium was dissected into pieces about 2.0 x 0.3 x 0.3 cm in size. The long axis of each strip was cut in the direction of the muscle bundles. The dissection was done in a petridish containing Tyrode's solution bubbled with a gas mixture of 95 % oxygen and 5 % carbon dioxide.

Instruments

1. Organ bath (double walled Churchill type)
2. Thermostatic bath
3. Dynograph (Beckman type RM)
4. Isotonic transducer (Statham UC 3)
5. Blender
6. Rota vapor
7. Thin-layer chromatography

Garlic and Chemicals

1. Garlic cloves purchased from the market
2. Chloroform (Standard Lab)
3. Povidone (PVPK)
4. D (+) - Glucose - Monohydrate (Merck)
5. Sodium chloride (NaCl, Merck)
6. Sodium dihydrogenphosphate ($NaH_2PO_4 \cdot 2H_2O$, Merck)
7. Sodium hydrogen carbonate ($NaHCO_3$, BDH)
8. Calcium chloride ($CaCl_2 \cdot 2H_2O$, Merck)

9. Magnesium chloride ($MgCl_2 \cdot 6H_2O$, M & B)
10. Potassium chloride (KCl, BDH)
11. Atropine sulfate (Sigma)
12. Phentolamine (Ciba - Geigy)
13. Propranolol hydrochloride (Sigma)
14. Verapamil (Knoll)
15. Nifedipine (Bayer)

Preparation of Allicin

1. Extraction of Allicin

The procedures of allicin extraction were followed those described by Poolsanong (1984). Garlic cloves purchased from the market were used for allicin extraction. The dry outer scales were removed. 100 gm of the cloves was washed and dried. 120 ml of chloroform was added and thoroughly blended until a good mixture was obtained. Then the mixture was filtered through a four-layer fine muslin cloth and through Whatman filter-paper No. 1, respectively. Chloroform was separated from the filtrate by Rota vaporization at 55°C and the yellowish oily liquid was obtained. The extract was examined for allicin at relative flow of 0.74 (0.70 - 0.75) by thin-layer chromatography. The allicin extract was then preserved with 1.2 gm of povidone and was stored in a refrigerator. This preservation made it stable for about 1 year. By this extraction method, 5 % of allicin was obtained. Diagram of the procedures of allicin extraction is shown in Fig. 2.

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2. Dilution of Allicin Preparation

One ml of allicin extract (2,000 mg/ml) was suspended in 49 ml of normal saline solution and then thoroughly stirred. The concentration of the allicin suspension, therefore, was 40 mg/ml.The suspension was then diluted in normal saline solution to make a final concentration of 4 mg/ml and stored in a refrigerator for subsequent use. After one-year extraction, quantitative analysis of allicin by gas chromatography showed that the concentration was 3.6 mg/ml, which was decreased by 10 %.

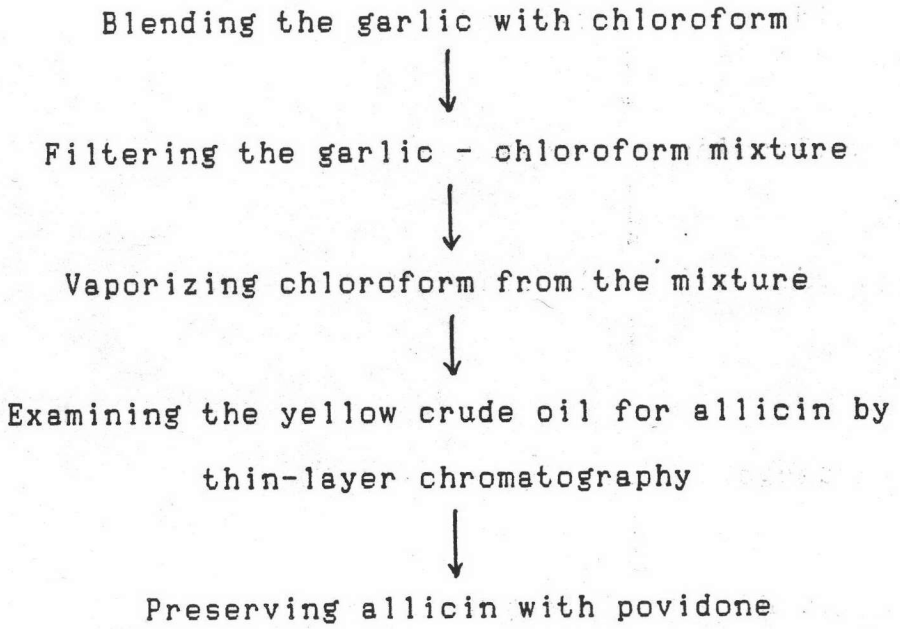


Fig. 2. Diagram shows the procedures of allicin extraction.

Preparation of Tyrode's Solution

Tyrode's solution was used as physiological solution for the study of human myometrial contraction (Lohsiriwat and Anumanrajadhon, 1986). The solution was prepared by dissolving the chemicals in distilled water. The composition is shown in Table 3.

Table 3. Composition of Tyrode's Solution.

| Chemicals | Weight (gm/L) |
|---|---|
| NaCl | 8.00 |
| KCl | 0.20 |
| $CaCl_2$ | 0.10 |
| $MgCl_2$ | 0.10 |
| $NaHCO_3$ | 1.00 |
| $NaH_2PO_4$ | 0.05 |
| Glucose | 1.00 |
| pH = 7.4 | |
| aerating gas = 95 % $O_2$ + 5 % $CO_2$ | |

Experimental Procedures

One end of the muscle strip was sewn by thread No. 60 and attached to a glass hook held in an organ bath (Fig. 3) containing 20 ml of Tyrode's solution thermostatistically controlled at a temperature of 37°C and bubbled with a gas mixture of 95 % oxygen and 5 % carbon dioxide. The other end of the muscle strip was tied to a displacement force transducer (Statham UC 3). The contractile activity was recorded isotonically (Fig. 3) on a dynograph (Beckman type RM). The initial load was set at 2 gm. In every experiment, the tissue was allowed to equilibrate in the bath for 30 minutes and washed three times with the solution every 15 minutes. The spontaneous contractile activity was recorded after the equilibration.

The experiments were performed as the followings:

1. Study of the effects of allicin on the contraction of isolated human uterine muscle during proliferative phase of the menstrual cycle.

Six doses of allicin 0.2, 0.3, 0.4, 0.5, 0.6 and 0.8 ml of 4 mg/ml were tested in the experiments. The tissue was washed after each treatment. Contractile responses to various doses of allicin were recorded in terms of force, rate and form.

2. Study of the mechanism of action of allicin.

The following blockers were used as pretreatment : atropine sulfate as a muscarinic antagonist, phentolamine as an alpha-adrenergic antagonist, propranolol as a beta-adrenergic antagonist, verapamil and nifedipine as calcium blocking agents, at the concentration of $10^{-4}$, $10^{-4}$, $10^{-4}$, $10^{-5}$ and $10^{-5}$ M, respectively. Only one antagonist was used on each piece of tissue. 0.4 ml of allicin (4 mg/ml) was then added to the bath after each pretreatment. Contractile responses to allicin in the presence of various blockers were recorded.

Statistical Analysis

The results were presented as mean ± SEM. Student's unpaired t - test was used to evaluate the levels of significant differences between all of the mean values. The probability values that were less than 0.05 were considered to be significant.

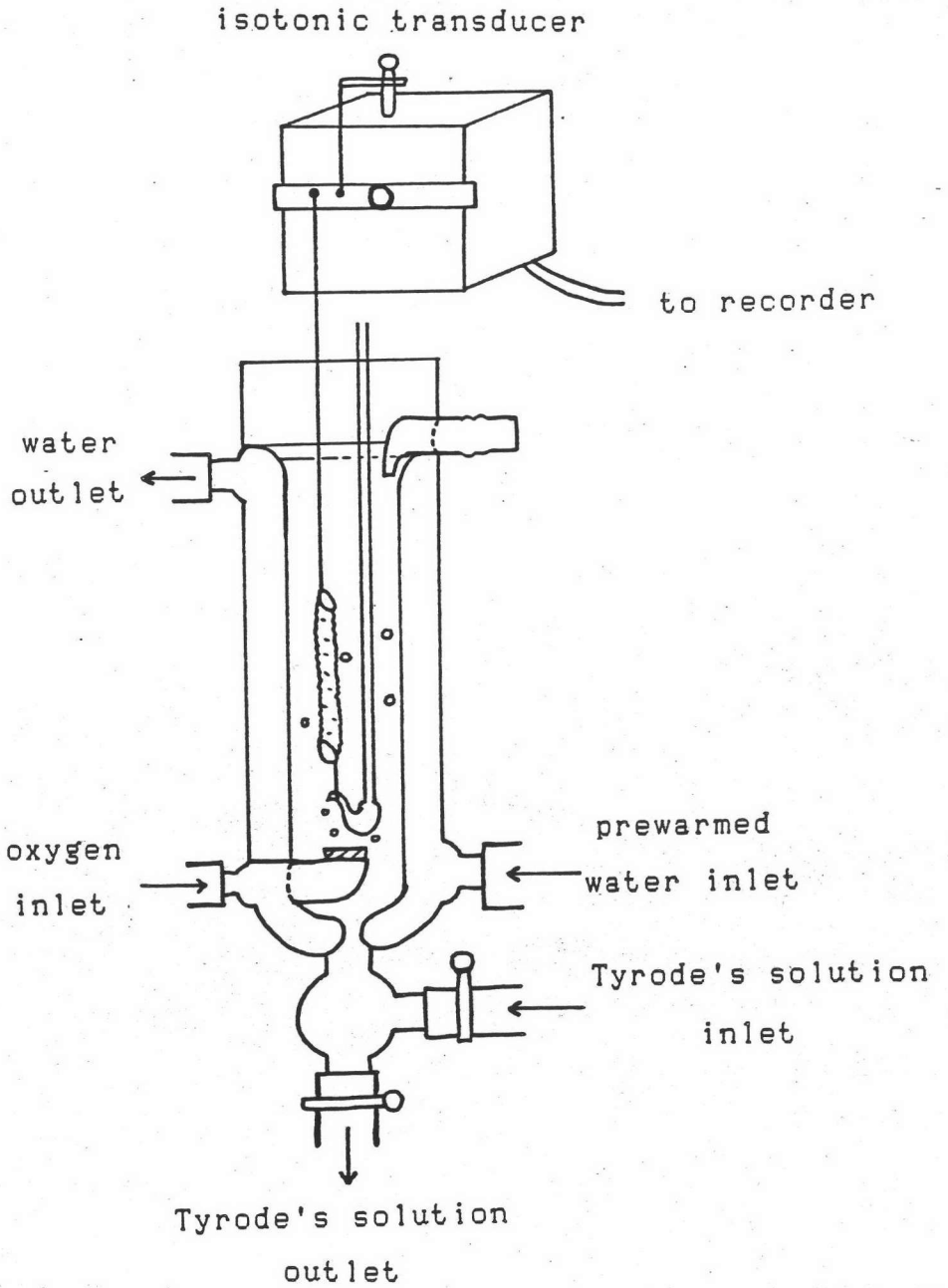


Fig. 3. Schematic diagram of isolated organ bath preparation. The contractile activity of a strip of human myometrium was isotonically recorded with a Dynograph in terms of force, rate, and form.