

**SIBLIOGRAPHIC DATA SHEET**1. CONTROL NUMBER  
PN-AAH-8072. SUBJECT CLASSIFICATION (695)  
PC00-0000-0000

## 3. TITLE AND SURTITLE (240)

Research on prostaglandins in relation to human reproduction; annual report, 1974

## 4. PERSONAL AUTHORE (100)

## 5. CORPORATE AUTHORS (101)

Singapore Univ.

## 6. DOCUMENT DATE (110)

1974

## 7. NUMBER OF PAGES (120)

12p.

## 8. ARC NUMBER (170)

SN613.943.S617-1974

## 9. REFERENCE ORGANIZATION (130)

Singapore

## 10. SUPPLEMENTARY NOTES (500)

## 11. ABSTRACT (950)

## 12. DESCRIPTORS (920)

Birth control  
Family planningFertility  
Prostaglandin

## 13. PROJECT NUMBER (150)

932060200

## 14. CONTRACT NO.(140)

AID/CM/pha-0-73-36

15. CONTRACT  
TYPE (140)

## 16. TYPE OF DOCUMENT (160)

52

SN  
613.943  
S617  
Sept. 1974

PN-AAH-807

Sept 14, 1974

U.S.A.I.D. CONTRACT NO. AID/CM/pha-C-73-36

RESEARCH ANNUAL REPORT

Sultan M. M. Karim - Principal Investigator and  
Project Director, Research Professor of Obstetrics  
and Gynaecology, University of Singapore, Singapore.

A.I.D.  
Reference Center  
Room 1656 NS

General background and Project objective:

The objective of this contract is the accomplishment of a research program essential to the further development and testing of the value of prostaglandins as abortifacients and as a means of fertility control. The program, in accomplishment of the objective, will involve an investigation into the Physiological roles, pharmacological actions and clinical applications of some naturally occurring Prostaglandins and their synthetic analogs. New formulation and routes of administration will be developed and tested. Special studies on the safety of prostaglandins will examine their effects on cardiovascular, gastrointestinal, haematological, endocrine and ocular systems. The evaluation of the physiological roles of prostaglandin in relation to human reproduction and their mechanism of action will involve the measurements of Prostaglandins, steroid hormones (estrogens, progesterones), L. H. , F. S. H. and H. C. G. in pregnant and non-pregnant subjects. The program will allow for an extension and continuation of clinical trials of Prostaglandin as a means of pregnancy termination and as a once-a-month means of fertility control. To disseminate prostaglandin technology and methods, fellows from other countries will be trained and collaborative clinical trials of these substances as abortifacients and contraceptives will be conducted.

Laboratories, Equipment and Personnel Recruitment

Limited laboratory facilities existed in the Department of Obstetrics and Gynaecology prior to the commencement of the project. However these facilities had to be extended. The University assisted in providing additional laboratories which have now been refurnished and functioning. The following laboratories have been set up.

1. Physiology / Pharmacology Laboratories
2. Biochemistry / Radioimmunoassay Laboratories
3. Haematology Laboratories
4. Tissue Culture Laboratories
5. Animal House and Animal Experiments Laboratories
6. Baboon House

### I. Physiology / Pharmacology Laboratories

The following facilities have been established to study pharmacological effects and to evaluate Physiological functions of Prostaglandins.

#### (a) Isolated tissue Laboratory

Facilities are available to study effects of Prostaglandins (and other drugs) on a variety of isolated tissues from animals and man including uterus, fallopian tubes, bronchial muscle, bladder, gastrointestinal tract smooth muscle, umbilical and placental blood vessels.

#### (b) Intact animal

Facilities have been established to study effects of drugs on the cardiovascular, respiratory, gastrointestinal and reproductive systems of rats, rabbits, cats, dogs and baboon.

#### Work Performance

More than a dozen Prostaglandins are known to occur naturally. Of these only two ( $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$ ) have so far been studied for their abortifacient actions and for possible use for menstrual regulation. There are several reasons for exploring the potentials of other naturally occurring Prostaglandins for practical applications in the above areas.

(a) Most of the naturally occurring Prostaglandins have a stimulant action on isolated strips of pregnant human uterus.

(b) Prostaglandins of the A and B series are less readily metabolised and inactivated. They should therefore have a prolonged duration of action requiring less frequent administration.

(c) Some naturally occurring prostaglandins are thought to be relatively inactive in stimulating gastrointestinal tract smooth muscle when compared with  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$ . The use of such compounds could overcome nausea, vomiting and diarrhoea associated with the use of  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  as abortifacient.

(d) Some of the naturally occurring Prostaglandins although less potent than  $\text{E}_2$  and  $\text{F}_{2\alpha}$  in stimulating the uterus may act as abortifacients by mechanisms other than direct uterine muscle stimulation e. g.

(i) by constricting umbilical and placental blood vessels or

(ii) by interfering with the production of hormones necessary for the maintenance of pregnancy.

As a preliminary to exploring the use of some naturally occurring Prostaglandins as abortifacients Pharmacological studies in the following area are being undertaken.

(a) Effect on isolated human umbilical blood vessels.

(b) Effect on isolated human and animal bronchial muscle (because of the known bronchodilator effect of PGF compounds).

(c) Effect on isolated human uterus and fallopian tubes.

(d) Effect on the cardiovascular, gastrointestinal and respiratory systems in the baboon (from previous studies it is known that the baboon is a good model for studying these effects of PGs).

(e) Effect on the intact uterus in the baboon (including abortifacient and luteolytic action).

(f) Acute toxicity in the baboon.

### Umbilical Cord blood vessels

The constrictor action of Prostaglandins  $E_2$ ,  $F_{2\alpha}$  and  $F_{1\alpha}$  on isolated umbilical blood vessels has been previously reported. In an investigation carried out under the contract the effects of  $PGA_1$ ,  $A_2$ ,  $B_1$ ,  $B_2$  on isolated human umbilical blood vessels has been investigated and compared with  $PGE_2$  and  $F_{2\alpha}$ . The potencies in decreasing order are  $PGA_2 > B_2 > F_{2\alpha} > B_1 > E_2 > A_1$ . Detailed results in the form of a manuscript are enclosed.

### Acute toxicity of Prostaglandins $E_2$ , $F_{2\alpha}$ and 15 (S) 15 methyl $E_2$ methyl ester in the baboon

Prostaglandins  $E_2$ ,  $F_{2\alpha}$  and 15 (S) 15 methyl  $E_2$  methyl ester given by intra-amniotic route are used for the termination of second trimester pregnancy. In spite of the high doses employed this procedure is associated with fewer side effects compared with the systemic administration of these compounds because of slow diffusion across the fetal membranes to the uterus. However inadvertent systemic injection of the doses of PGs intended for intra-amniotic administration would be expected to produce serious side effects in view of the known pharmacological effects of much smaller doses of these compounds. A study was therefore undertaken to determine the lethal intravenous dose of Prostaglandins in the baboon. For  $PGE_2$  the lethal dose was found to be 64 times higher than the intra-amniotic abortifacient dose and for  $PGF_{2\alpha}$  it was 256 times. Single intravenous dose of 15 (S) 15 me  $E_2$  me ester. 80 times higher than the intra-amniotic abortifacient dose did not prove lethal in the baboon. There are reasons to believe that the data obtained from baboon studies are applicable to the human (see enclosed paper for further details).

### Effect of human bronchial smooth muscle in vitro

Occasionally, the use of  $\text{PGF}_{2\alpha}$  for termination of pregnancy is associated with bronchospasm. For this reason it is essential to evaluate the effect of any 'new' PG intended for clinical use on the bronchial smooth muscle. From preliminary studies on isolated human bronchial muscle (obtained at surgery or from fresh post-mortem cases) the following data has been accumulated.

1.  $\text{PGF}_{1\alpha}$  is 20 times less potent than  $\text{PGF}_{2\alpha}$  in its effect on bronchial smooth muscle.
2.  $\text{PGB}_1$  is half as active as  $\text{PGF}_{2\alpha}$ .
3.  $\text{PGB}_2$  is twice as potent as  $\text{PGF}_{2\alpha}$ .
4.  $\text{PGA}_1, \text{PGA}_2$  and  $\text{PGF}_{2\beta}$  have a relaxant effect on human bronchial muscle.

Thus as far as the bronchial effect is concerned  $\text{PGF}_{1\alpha}$  in doses 20 times higher than  $\text{PGF}_{2\alpha}$  can be safely administered. The high bronchoconstrictor potency of  $\text{PGB}_2$  would be a limiting factor in its clinical use.

### Acute Cardiovascular studies in the baboon

$\text{PGF}_{2\alpha}$  and  $\text{PGB}_2$  increase and  $\text{PGE}_2, \text{A}_1, \text{A}_2, \text{B}_1, \text{F}_{1\alpha}$  decrease arterial blood pressure in man. The baboon responds in a similar manner (both qualitatively and quantitatively) to prostaglandins. The data obtained are summarised below:

$\text{PGE}_2$  is 5 times more potent than  $\text{PGF}_{1\alpha}$  in lowering arterial blood pressure in anaesthetised baboon.

$\text{PGE}_2$  and  $\text{PGA}_1$  and  $\text{A}_2$  are equipotent.

$\text{PGE}_2$  is 50 times more potent than  $\text{PGB}_1$ .

$\text{PGF}_{2\alpha}$  is 4 times more potent than  $\text{PGB}_2$  in raising arterial blood pressure in the baboon.

Effect of Prostaglandins on the pregnant human uterus in vitro

Apart from  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  all other naturally occurring Prostaglandins have a stimulant action on strips of pregnant myometrium obtained at caesarean section. These include  $\text{PGA}_1$ ,  $\text{A}_2$ ,  $\text{B}_1$ ,  $\text{B}_2$  and  $\text{F}_{1\alpha}$ .

Physiological/Pharmacological studies to be carried out during the 2nd year of the project

The studies outlined above will be continued and completed during the second year of the project. Additional studies in the following areas will be carried out :

Quantitative studies of potencies of several naturally occurring Prostaglandins (relative to  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$ ) on (a) isolated human uterus (b) on the pregnant and non-pregnant baboon uterus in vivo (c) abortifacient potencies in the baboon, will be carried out.

II. Biochemistry/Radioimmunoassay Laboratories

Radioimmunoassay

The following radioimmunoassays have been set up:

- a) Prostaglandins  $\text{F}_{2\alpha}$ , 15-keto  $\text{F}_{2\alpha}$ ,  $\text{E}_2$ ,  $\text{A}_2$  and  $\text{B}_2$ .
- b) Progesterone
- c) estrogens
- d) Human chorionic gonadotrophins
- e) follicle stimulating hormone
- f) Luteinizing hormone
- g) Human Placental lactogen

specific antisera against prostaglandin, progesterone, hCG and hPL are being raised by us.

### Gas-Liquid Chromatography

Prostaglandins are also being assayed by gas-liquid chromatography.

### Work Performance

- a) Control data on plasma levels of L.H., F.S.H., Progesterone and estrogens in women during normal menstrual cycle have been obtained.
- b) Plasma levels of Progesterone, estrogens, hCG, hPL during different stages of pregnancy have also been determined.

### Work to be carried out during the second year of the Contract

The effect of naturally occurring prostaglandins and synthetic analogs on steroid and protein hormones will be determined in the following groups of patients:-

- a) Prostaglandins given during the luteal phase of the menstrual cycle to investigate a possible luteolytic effect.
- b) Prostaglandins administered for menstrual regulation (during the first 15 days of missed menses) to investigate if the mechanism of action is through (i) luteolytic effect or, (ii) through direct uterine muscle stimulation.
- c) Prostaglandins administered by different routes for the termination of 1st and 2nd trimester pregnancy.

### III. Tissue Laboratories

During the past six months efforts have been diverted towards developing in vitro systems for the investigation of Steroidogenic effects of Prostaglandins in human tissues. Two different experimental approaches have been employed:

- a) Steroid hormones biosynthesis in tissue homogenates
- b) Steroidogenesis in cells grown in tissue culture.

### Tissue Homogenates

The importance of placental steroidogenesis is well established. Preliminary studies have been carried out to establish the optimum conditions for the in vitro synthesis of progesterone in the tissue homogenates of full term placenta. Radioimmunoassays are employed to measure progesterone in this system. Column and two dimensional thin layer chromatographic methods are used for the purification and characterization of the newly synthesized progesterone. It has been confirmed that tissue homogenates from human placenta are capable of synthesizing progesterone from endogenous precursors. The synthesis is stimulated by NAD- and NADPH-generating systems.

A tissue culture laboratory to provide facilities for growing human ovarian cells in culture has been set-up.

### Work to be carried out during the second year of Contract

- a) Tissue homogenate studies will be extended to study luteal steroidogenesis in the human corpus luteum.
- b) Granulosa cell cultures will be used to study the process of transformation of these cells into luteal cells.
- c) The effect of naturally occurring Prostaglandins and some synthetic analogs on progesterone synthesis in tissue homogenates of placenta and corpus luteum will be studied.
- d) Granulosa and corpus luteum cells in culture will be studied to determine the effect of Prostaglandins on the synthesis of progesterone.

#### IV. Haematology Laboratories

One of the last laboratories to be set-up. The laboratory can now study the following haematological parameters:

Prothrombin time

Kaolin Cephalin Clotting time (KCCT)

Thrombin time

Ethanol gel test

Fibrinogen titre

Platelet count

Coagulation Factors I, s, 5, 7, 8 and 10

Antithrombin III

Anti Xa

2 macroglobulin

Fibrinogen-Fibrin degradation products (F. D. P.)

Platelet aggregation

Platelet adhesiveness

Heparin assay

Thromboelastography

These facilities will in future be utilized to study haematological effects of Prostaglandins.

#### Clinical Studies

During the discussion of the project and prior to the signing of the Contract it was appreciated by both sides that to achieve further progress in clinical uses of PGs, synthetic compounds will have to be used. Accordingly approval was sought from USAID to introduce Prostaglandins 15 (S) 15 methyl E<sub>2</sub> me ester into clinical trial. This approval was contained in your letter of 8th April (received 17th April 1974). An unexpected problem was encountered in obtaining adequate supplies of this analog. Attempts to purchase the compound from Pharmaceutical companies failed. Eventually we have managed to obtain sufficient of the analog (gratis) to commence clinical studies. For reasons outlined above it has not been possible to carry out any clinical studies during the first year of the contract.

Starting August this year the following clinical studies have been commenced which we hope to complete during the second year of the contract.

1. Pre-operative cervical dilatation

As a result of a study carried out from other grants, it has been established that extraovular administration of a single dose of 25 µg 15 (S) 15 methyl E<sub>2</sub> methyl ester given 12-14 hours prior to D & C, is effective in dilating the cervix. A reprint of paper is attached. The study was carried out in patients of all parity and at different stages of gestation. It is realised that pre-operative dilatation of the cervix is more useful in nulliparous women than in multiparous women. As a result the following study has just been started:

1) A double blind study on the effectiveness of a single extraovular dose of 25 µg 15 (S) 15 methyl E<sub>2</sub> methyl ester in 25 nulliparous women during 8-13 weeks gestation. Control group will consist of 25 nulliparous women who will receive blank vehicle. Informed consent is being obtained from all patients. The trial will be extended to a larger group of women (depending upon the results of the preliminary study).

2) Termination of late first trimester and early second trimester pregnancy

Naturally occurring PGs given by the extraovular route are effective in terminating late first trimester and early second trimester pregnancy. However doses of PGs have to be repeated every 1-2 hours. Prostaglandin analog 15 (S) 15 me E<sub>2</sub> methyl ester in a dose of 30 µg administered extraovularly stimulated the uterus for 8-10 hours.

The study will be carried out in 25 women in the late first trimester and early second trimester of pregnancy. PG analog in a dose of 30 µg extraovularly will be given at 8-10 hourly interval.

3) Intra-amniotic administration of 100 µg 15 (S) 15 methyl E<sub>2</sub> methyl ester is being studied for the termination of 2nd trimester pregnancy.

4) Menstrual regulation

A study to explore the use of intrauterine administration of 25 µg of 15 (S) 15 me E<sub>2</sub> me ester for menstrual regulation (delay in menstruation of up to 2 weeks) will also be carried out during the second year of the contract.

## Personnel

The following personnel are engaged in the performance of the work under the Contract.

1. Dr. Sultan M.M. Karim  
Principal investigator and Project director  
(Salary paid by the University of Singapore)
2. Dr. S.S. Ratnam  
Clinical Co-ordinator (salary paid by the  
University of Singapore)
3. Dr. John A. Salmon  
Research Fellow (salary paid of Dr. Karim's  
personal research grant)
4. Dr. B. Rao  
Reproductive Biologist - Research Fellow
5. Dr. V. Bhandari  
Clinical Assistant
6. Mrs. Chow Mei Tong  
Research Assistant
7. Mrs. Ng Boon Cheng  
Research Assistant
8. Miss Lo Pia Yǒng  
Research Assistant
9. Mr. P.A. Ganesan  
Research Assistant (salary paid out of  
Dr. Karim's personal research grant)
10. Miss Leong Yun Kiew  
Laboratory Technician
11. Miss Tan Swee Chui  
Laboratory Technician
12. Miss Siew Cheow Hiah  
Laboratory Technician
13. Mrs. Loi Lee Boon  
Research Nurse
14. Mrs. Wong Weng Kit  
Research Nurse
15. Miss Gurdip Kaur  
Laboratory Attendant
16. Mr. Nallathambi s/o Karrupiah  
Laboratory Attendant
17. Mr. Johari bin Jani  
Laboratory Attendant
18. Mr. Thamsegaren s/o Thar'maungam  
Laboratory Attendant
19. Mr. M. Mari Muthu  
Driver
20. Miss Lily Kch  
Secretary