THE ROLE OF THE HORMONE RELAXIN

IN HUMAN REPRODUCTION

A THESIS SUBMITTED BY

ALASTAIR HARVEY MACLENNAN

M.B.Ch.B., F.R.C.O.G., F.R.A.C.O.G.

for the degree of

Doctor of Medicine

at the

University of Glasgow, Scotland.

The research described in this thesis was conducted at
The Department of Obstetrics and Gynaecology,
The University of Adelaide, South Australia.

Thesis submitted March 1985

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ACKNOWLEDGEMENTS

Inspiration I wish to firstly thank Professor's Fred and Gillian Greenwood for their enthusiasm, expertise and support in establishing my interest and laboratories in this field when they visited Adelaide in 1978 on their sabbatical leave from Hawaii. They have continued to support me and collaborate with me across the Pacific Ocean and I am indebted to them for their help and friendship.

Supervision Professor Lloyd Cox, Chairman, Department of Obstetrics and Gynaecology, The University of Adelaide, has been the official supervisor of this thesis. I am very grateful to him for his encouragement to write this thesis at this point in the relaxin story and for all the facilities of his department which he readily made available for this research. May thanks also to Dr. Robert Seamark, Reader in Reproductive Endocrinology, The University of Adelaide, who helped me through the initial technical problems in setting up the purification process for porcine relaxin and its radioimmunoassay.

Grants The majority of these studies was supported by a grant from the National Health and Medical Research Council of Australia. Grants were also later gratefully received from The Queen Victoria Research Foundation and The Queen Victoria Hospital's Women's Auxiliary.

Co-authors Whilst the candidate was first and main author on 15 of the papers (published or in press) I wish to acknowledge the help of my co-authors, particularly Sister Ros Green (Research Sister, The Queen Victoria Hospital) who chaperoned me through all the clinical trials and was responsible for many of the blood collections, Mrs. Patricia Grant (Technical Assistant, The University of Adelaide) who latterly

inherited much of the labour involved in the purification of the relaxin and muscle experiments and Mrs. Ruth Nicolson (N.H. and M.R.C. Research Assistant) who helped establish and run the radioimmunoassay.

Donations I am indebited to Dr. Elizabeth O'Byrne, New York, for supplying the R6 rabbit anti-porcine relaxin serum, and Dr. Christian Schwabe for donating the relaxin tracer. The relaxin standard was kindly supplied by the National Institutes of Health, U.S.A.

Advice Mr. Dietmar Ness, Research Officer, Department of Obstetrics and Gynaecology, The University of Adelaide, provided expert statistical advice and Dr. Helen Chambers, Senior Pathologist, The Queen Victoria Hospital, kindly reviewed my morphological studies in Chapter 14.

Illustrations Mr. Kym Tank, Clinical Photographer, The Queen Victoria Hospital, expertly printed most of the illustrations and I thank Miss Mary Ciganovic for her help with the art work.

Typing This thesis was painstakingly typed and retyped without complaint by Miss Carmel Sandercock and I thank her for sharing the work by compiling this thesis.

Support The doctors and nursing staff of The Queen Victoria Hospital have always been most generous in allowing me access to their patients and without their help and the generosity of the patients themselves, this research would not have been possible. My special thanks to my wife Alice for her understanding as to why I did not come home at night and for her help in the proof reading. Finally, I thank my two daughters Suzanna and Lorna for access to their guinea-pig, Marmalade,

who did confirm that several days prior to parturition the pubic symphysis does indeed relax.

SUMMARY OF THESIS

Relaxin is a polypeptide hormone, similar in structure to insulin, which was originally discovered 60 years ago as a hormone that relaxed the pubic symphysis of the guinea-pig prior to parturition. However, relaxin has only very recently been purified and characterized in the rat, pig and human. Its main source appears to be the corpus luteum of pregnancy but it is also produced by decidual tissue and the prostate gland. Its main mechanism of action appears to be the facilitation of remodelling of connective tissue in target tissues to allow the necessary changes in organ structure during pregnancy and parturition. A secondary mode of action in some mammals may be to inhibit uterine contractility until near the end of pregnancy. Until recently the existence of relaxin in the human was debated and its role was unknown. Human relaxin is not yet available for laboratory or clinical trials but it is now possible to purify large quantities of pure porcine relaxin.

The aim of this thesis was to examine the possible roles of relaxin in human reproduction, specifically in the areas of implantation of the fertilised ovum, the maintenance of myometrial quiescence during pregnancy and the facilitation of cervical ripening and parturition. These studies involved the extensive purification of porcine relaxin for laboratory experiments and for the first clinical trials of a pure relaxin. A homologous porcine relaxin radioimmunoassay was also set up to measure peripheral serum relaxin-like immunoactivity in normal and abnormal human pregnancy. Where possible human studies were performed but where this was not practically or ethically possible, in vitro or animal studies were performed to further elucidate relaxin's possible role in reproduction.

In a cross sectional study of 330 normal singleton human

pregnancies serum relaxin concentrations were found to be highest in early pregnancy and thereafter they gradually declined until term. Post-term patients had significantly lower relaxin concentrations and for the first time in the human a significant elevation in relaxin levels was described in early labour corresponding with such an elevation near parturition in most other animals studied. This pattern of relaxin secretion is in keeping with its postulated roles of maintaining uterine quiescence but with a waning influence until term and a cervical ripening action around parturition.

Serum relaxin levels were also measured in pathological pregnancies and for the first time a correlation has been made between pelvic joint pain and laxity in pregnancy and high relaxin concentrations. Relaxin levels were also high in late pregnancy in multiple gestations. However, low levels were found in patients in premature labour suggesting the possibility that such low levels might contribute to this pathology. Little maternal relaxin appears to cross into the fetal circulation and this assay could not detect high levels of relaxin in the umbilical cord blood of babies with congenital dislocation of the hip. Relaxin levels were not altered in pre-eclampsia.

In a study of the effect of a continuous infusion of porcine relaxin in rats during the time of implantation normal spacing and orientation of the blastocysts was disturbed and implantation sites were found to be confined to the cranial half of each uterine horn. In an attempt to utilise this effect to increase the pregnancy rate at human embryo transfer in an <u>in vitro</u> fertilisation programme pure porcine relaxin was given vaginally in a randomised controlled clinical trial. Such treatment did not improve or inhibit the pregnancy rate and a role for relaxin in implantation in the human could not be substantiated.

The *in vitro* effect of pure porcine relaxin on non-pregnant and pregnant myometrium before and after progesterone treatment was studied in the rat, pig and human. Porcine relaxin inhibited rat myometrial activity until mid pregnancy but had no effect in late pregnancy. Porcine relaxin inhibited pig myometrial activity throughout pregnancy but in contrast had no significant effect on human myometrial activity in the pregnant or non-pregnant state. Progesterone was synergistic with relaxin in the rat and pig when the latter hormone exhibited an inhibitory effect. It is suggested that the lack of response of human myometrium to porcine relaxin is due to the difference in structure between porcine and human relaxin rather than there being a lack of relaxin receptors on human myometrium.

In a series of randomised controlled clinical trials pure porcine relaxin was shown for the first time to promote cervical ripening in patients near term and to induce labour in up to a third of those treated. Patients receiving porcine relaxin had a significantly shorter mean length of labour and less analgesic requirements. The clinical effects of vaginal and intracervically applied relaxin were similar to the effects of prostaglandin $F_2\alpha$ in other clinical trials of cervical ripening conducted by the candidate. Further clinical and morphological studies described in this thesis concluded that prostaglandin $F_2\alpha$ and relaxin appear to activate the same collagenolytic system rather than acting in parallel to produce separate connective tissue changes. No side effects were detected following the use of pure porcine relaxin in any of the clinical trials and the induction of porcine relaxin antibodies was not detected. Porcine relaxin was shown to be systemically absorbed from the lower human genital tract and the association of both high endogenous and exogenous relaxin levels with cervical ripening and parturition suggests a role for relaxin in this aspect of human reproduction.

Finally a hypothesis is presented as to the role of relaxin and its controlling mechanisms in the maintenance of uterine quiescence during pregnancy and the facilitation of parturition at term. The synthesis of human relaxin is awaited to test this hypothesis and further substantiate the apparent important role of relaxin in human reproduction.

LIST OF PUBLICATIONS INCLUDED IN THIS THESIS

1. MacLennan, A.H. (1983) Invited review. The role of relaxin in human reproduction. Clinical Reproduction and Fertility, 2, 77-95.

2. MacLennan, A.H., Nicolson, R. & Green, R.C. (1985) Serum relaxin levels in normal human pregnancy. Submitted to Australian and New Zealand Journal of Obstetrics and Gynaecology.

3. MacLennan, A.H., Nicolson, R., Green, R.C. & Bath, M. (1985) The relationship of the hormone relaxin to pelvic pain and joint laxity in human pregnancy. Submitted to Australian and New Zealand Journal of Obstetrics and Gynaecology.

4. MacLennan, A.H., Nicolson, R. & Green, R.C. (1984) Relaxin levels in multiple pregnancy, premature labour, pre-eclampsia, normal neonates and neonates with congenital dislocation of the hip. In Proceedings of International Congress - Progress in Reproductive Medicine, Adelaide, South Australia.

5. Rogers, P.A.W., Murphy, C.R., Squires, K.R. & MacLennan, A.H. (1983) Effects of relaxin on the intrauterine distribution and antimesometrial positioning and orientation of rat blastocysts prior to implantation. Journal of Reproduction and Fertility, 68, 431-435.

6. MacLennan, A.H., Kerin, J.F.P., Kirby, C., Grant, P., Warnes, G. M., Cox, L.W., Bryant-Greenwood, G.D. & Greenwood, F.C. (1985) Accepted for publication by Australian and New Zealand Journal of

Obstetrics and Gynaecology.

7. MacLennan, A.H., Grant, P., Ness, D. & Down, A. (1985) The effect of porcine relaxin and progesterone on rat, pig and human myometrial activity in vitro. Submitted to American Journal of Obstetrics and Gynecology.

8. MacLennan, A.H., Green, R.C., Bryant-Greenwood, G.D., Greenwood, F.C. & Seamark, R.F. (1980) Ripening of the human cervix and induction of labour with purified porcine relaxin. Lancet, i, 220-223.

9. MacLennan, A.H., Green, R.C., Bryant-Greenwood, G.D., Greenwod, F.C. & Seamark, R.F. (1981) Cervical ripening with combinations of vaginal prostaglandin $F_2\alpha$, estradiol and relaxin. Obstetrics and Gynecology, 58, 601-604.

10. MacLennan, A.H., Green, R.C. & Grant, P. (1984) Ripening of the human cervix and induction of labour with intracervical purified porcine relaxin. Submitted to Obstetrics and Gynecology.

11. MacLennan, A.H., Katz, M. & Creasy, R. (1985) The morphology of cervical ripening induced by the hormones relaxin and prostaglandin $F_2\alpha$ in a rabbit model. Accepted by American Journal of Obstetrics and Gynecology.

12. MacLennan, A.H., Nicolson, R. & Green, R.C. (1985) The absorption of porcine relaxin from the human vagina and cervical canal. Submitted as a short communication to American Journal of Obstetrics and Gynecology.

13. MacLennan, A.H., Bryant-Greenwood, G.D. & Greenwood, F.C. (1983) Follow up studies on patients involved in clinical trials of porcine relaxin. In Biology of Relaxin and its Role in the Human, ed. Bigazzi, M., Greenwood, F.C. & Gasparri, F. pp 329-336. Amsterdam: Excerpta Medica.

14. MacLennan, A.H. & Green, R.C. (1979) Cervical ripening and induction of labour with intravaginal prostaglandin $F_2\alpha$. Lancet, i, 117-119.

15. MacLennan, A.H. & Green, R.C. (1980) A double blind dose trial of intravaginal prostaglandin $F_2\alpha$ for cervical ripening and the induction of labour. Australian and New Zealand Journal of Obstetrics and Gynaecology, 20, 80-83.

16. MacLennan, A.H. & Green, R.C. (1980) The effect of intravaginal prostaglandin F_2a on labour after spontaneous and artificial rupture of the membranes. Australian and New Zealand Journal of Obstetrics and Gynaecology, 20, 87-90.

CHAPTER 1

INTRODUCTION

1.1 THE AIM OF THE THESIS

The polypeptide hormone relaxin has only recently been confirmed to be present in the human and specific roles for it in the human have not been delineated. The main aim of this thesis is to examine possible roles for this hormone in the human specifically in the areas of implantation of the fertilised ovum, the maintenance of myometrial quiescence during pregnancy and the facilitation of cervical ripening at parturition. As human relaxin was not available at the beginning or during any of these studies, pure porcine relaxin and a porcine relaxin radioimmunoassay were used to investigate the likely role of relaxin in the human. These studies have involved newly developed methods to purify extensive amounts of pure porcine relaxin for laboratory and clinical trials and the development of a homologous porcine relaxin radioimmunoassay for its heterologous application in measuring relaxin-like immunoactivity in the human. Where ethically and practically possible the studies were designed to investigate the role of the hormone relaxin in the human using human samples or by conducting clinical trials. However, where this was not possible, animal or in vitro studies have been performed to further define relaxin's possible role in reproduction.

1.2 HISTORICAL REVIEW The Discovery of Relaxin

The presence of a hormone causing separation of the pubic symphysis of certain species of rodents before parturition was first detected by Frederick Hisaw in 1926 (Hisaw 1926) when he found that serum from pregnant guinea-pigs injected into virgin guinea-pigs caused relaxation of the pelvic ligaments. Such changes in the experimental animal could be induced only during oestrus or, as was later shown, after the animal was oestrogen-primed. Hisaw and his colleagues found that the corpus luteum of pregnancy was the main source of the factor causing relaxation of the public ligament (Fevold, Hisaw & Meyer 1930). From the corpora lutea of pregnant sows they obtained a fat-soluble extract and an aqueous extract. The fat-soluble extract induced progestational changes in the uterine endometrium and this extract was named 'corporin'. Corporin was later found to be a combination of the hormones oestrogen and progesterone. The aqueous extract of the corpora lutea contained the relaxing properties first noted by Hisaw, and it was proposed that the hormone in this extract be called 'relaxin'. As early as 1930, Fevold et al (1930) noted the similarity to insulin of relaxin's chemical features. However, for the next 30 years the purity of the relaxin extracts remained poor, approximately 90% of each preparation containing non-relaxin ovarian contaminants. These impure forms of relaxin were commercially produced under various commercial names, e.g. 'Releasin', 'Lutrexin', 'Cervilaxin'. The impurity of the hormone, together with difficulty in reproducing the bioassay (which used relaxin's effect on the guinea-pig public ligament) led to confusing and contradictory reports about its biological effects for several decades until much improved methods for isolating relaxin in its pure form were published in 1974.

1.3 THE ISOLATION AND PURIFICATION OF RELAXIN

Improvements in the purification of porcine relaxin began in the 1960s (Cohen 1963; Frieden 1963; Griss et al 1967) and in 1974 Sherwood and O'Byrne reported its biochemical characterization and purification, revealing three continuous peaks on elution from a carboxymethyl cellulose column. The relaxin preparations obtained from these peaks were called CMB, CMa and CMA, respectively. All three were shown to have similar biological potency in the mouse intrapubic ligament assay. It has since been possible to increase the yields of porcine relaxin from pregnant sow ovaries by inhibiting its degradation during purification by adding protease inhibitors to the ovarian extracts (Kwok, Bryant-Greenwood & Niall 1980). Recent studies (Walsh & Niall 1980) have shown that when proteolysis during purification is further dminished, CMa relaxin is the predominant relaxin found in vivo and that heterogeneity of the relaxin molecule appears to be due to loss of one or more amino acids during purification <u>in vitro</u> (Fig. 1). Walsh and Niall suggest that the major stored form of porcine relaxin <u>in vivo</u> is B31 relaxin (i.e. where relaxin's B chain contains 31 amino acids), and that the other molecular variants with shorter B chains are artefacts of the isolation procedures previously employed. Commercial production of relaxin using recombinant DNA technology is feasible and will be an important source of pure relaxin in the near future.

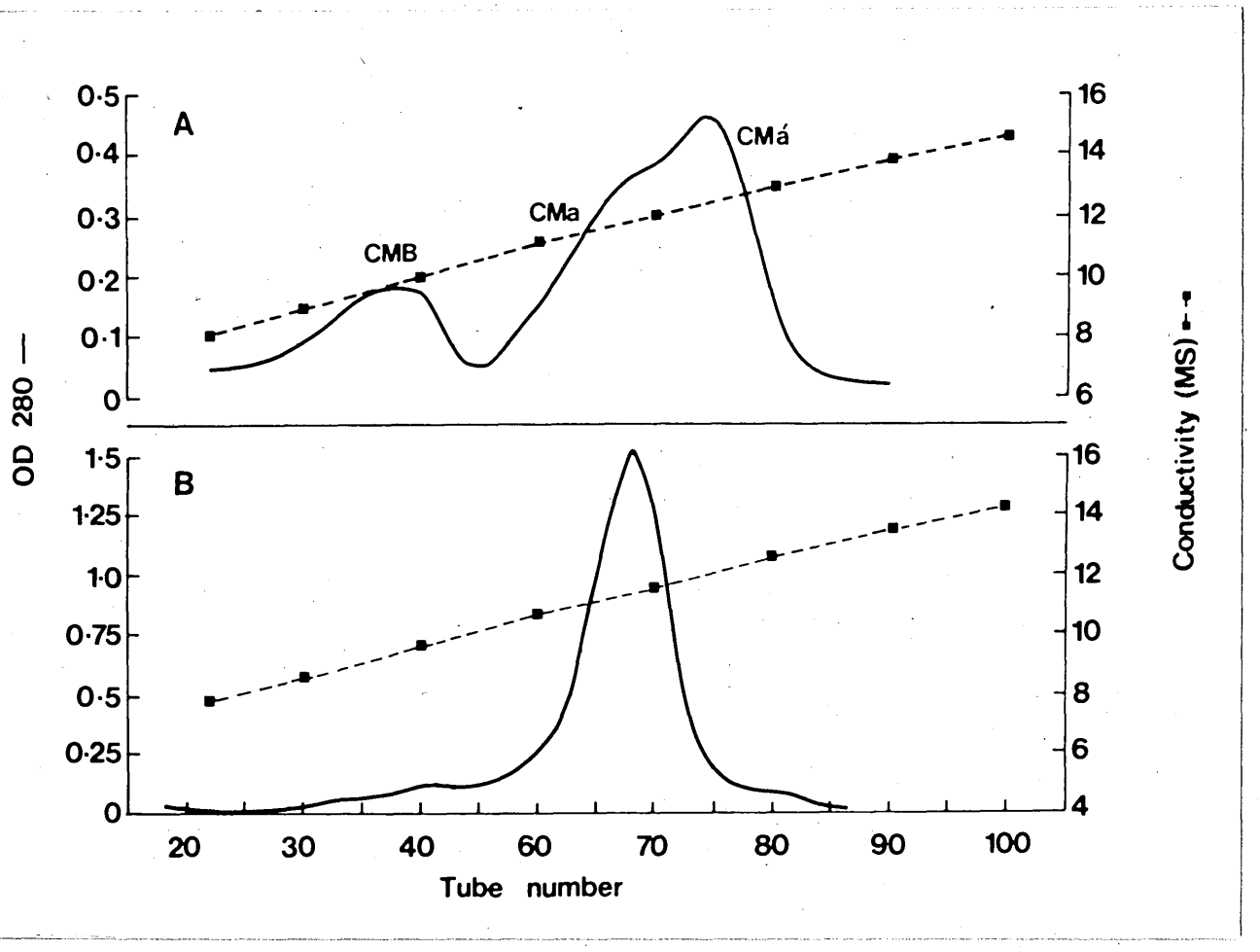


FIGURE 1 Carboxymethyl cellulose purification of porcine relaxin. (a) Procedure of Sherwood and O'Byrne giving three biologically active components, CMB, CMa and CMA, differing only in the length of their B chains (28-31 residues) due to proteolysis during purification. (b) Procedure of Walsh and Niall which markedly reduces proteolysis during the isolation process revealing only one relaxin peak which comprises mostly B31 relaxin (see text). Reproduced with permission from Walsh and Niall (1980).

1.4 BIOCHEMICAL STRUCTURE

Relaxin is a polypeptide hormone with a molecular weight of 6300. It has a conformational homology with insulin (Bedarkar et al 1977); both molecules have A and B chains connected by disulphide cross links in similar positions and share a similar tertiary structure. Approximately 25% of the amino acid sequences of insulin and porcine relaxin are identical (Niall 1979). Despite its similarity to insulin, no insulin-like biological activity has been described for relaxin (Schwabe & Harmon 1978). The complete structure of porcine relaxin was separately reported by Schwabe, McDonald & Steinetz (1976, 1977) and James et al (1977). These investigators published similar results for the A chain but there was disparity in the terminal sequence of the B chain. This disparity has now been resolved following the evidence by Walsh & Niall (1980) of the proteolysis during purification which accounted for the shorter chain described by Schwabe et al (1977). There is now evidence that one or more forms of a pro-hormone ('prorelaxin') occurs in vivo (Kwok, Chamley & Bryant-Greenwood 1979). Rat relaxin has recently been characterized (Sherwood 1979) and although similar in its tertiary structure, there are variations in its amino acid content.

The amino acid sequence of a human relaxin derived from the recent identification of a genomic clone for relaxin is now known (Hudson et al 1983). There is only approximately a 50% homology between porcine relaxin and this human relaxin (Fig. 2). The importance of this difference with regard to cross reactivity in vivo and in vitro is as yet unknown but porcine and human relaxins share the same tertiary structure and some of the same amino acid sequences at biologically active sites. At least one other relaxin-like gene may be present in the human (Hudson et al 1983). Absolute proof that the

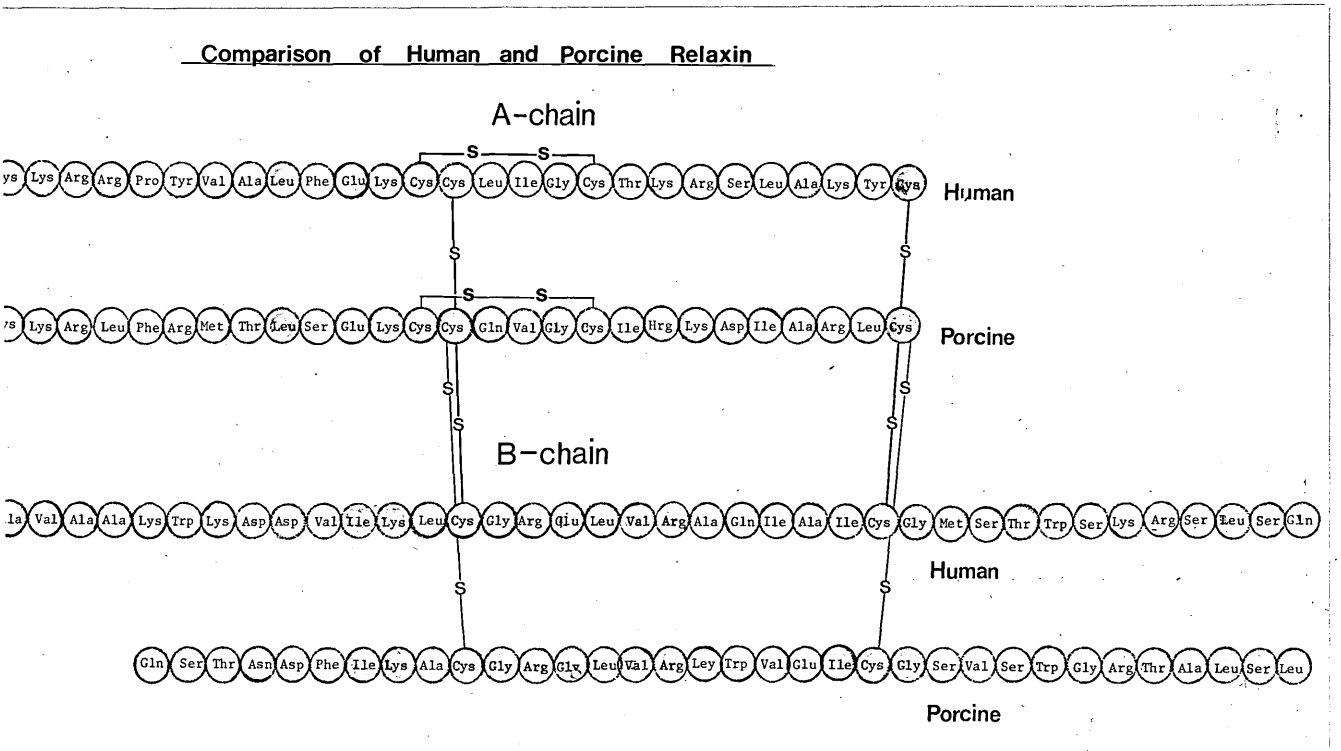


FIGURE 2 Comparison of the amino acid sequences of porcine relaxin and human relaxin. (Adapted from Hudson et al (1983)). Coloured circles denote amino acids in homologous positions and open circles represent dissimilar amino acids. S = Disulphide bonds.

above human genomic clone represents an expressed relaxin gene awaits identification of its corresponding cDNA derived from a relaxin secreting tissue in the human. Such tissues cannot easily be collected in the human but occasionally corpora lutea of pregnancy can be ethically collected and such work is presently under way. Sufficient amounts of human relaxin have not yet been purified or produced by genetic engineering techniques for any in vitro or in vivo studies.

Finally, relaxin and insulin share structural similarity to both nerve growth factor and insulin-like growth factor (James et al 1977). Thus, it seems probable that these four hormones have evolved from the same region of a primitive genome and therefore their biological actions may bear some comparison.

1.5 ASSAYS

1.5.1 Bioassays

During his early work, Hisaw simply classified samples as positive or negative for relaxin activity on the basis of their relaxational effect on the guinea-pig pubic symphysis. Abramowitz et al (1944) quantitated this effect, but the reproducibility of the test was poor. Subsequent work using more specific conditions improved the guinea-pig bioassay (Steinetz, Beach & Kroc 1969), but a mouse pubic symphysis assay has since been used with more success (Steinetz et al 1960). Nevertheless, bioassays remain laborious and are relatively insensitive. Although relaxin standards are available, the guinea-pig bioassay is not precise and the guinea-pig unit (GPU) has no precise quantitative meaning. Thus the biological activity of purified porcine relaxin (CM-A) can only be expressed as having a potency of approximately 2200 GPU/mg (Frieden et al 1980). More modern bioassays utilise the inhibitory effect of relaxin on oestrogen-primed rat myometrial activity (St. Louis 1981). These methods have been utilised in this thesis because of their greater reproducibility than pubic symphysis assays. They are described in Chapter 3.1.

1.5.2 Radioimmunoassays

The first radioimmunoassay for relaxin was described for porcine relaxin by Bryant in 1972 and was used to measure relaxin levels in various species. This assay utilized the labelling of tyrosine on a fraction of an impure relaxin preparation. When the structure of porcine relaxin was later characterized (Sherwood & O'Byrne 1974), pure relaxin was found not to contain

tyrosine. Thus, the first radioimmunoassay may have measured the prohormone or other relaxin-related compounds. Sherwood, Rosentreter & Birkhimer (1975) labelled pure porcine relaxin by the addition of tyrosine to the relaxin molecule and then radioiodinated the resulting polytyrosyl relaxin. Other methods of labelling relaxin for radioimmunoassay have since been described (Bryant-Greenwood & Greenwood 1979). Satisfactory correlation of radioimmunoactivity and biological activity has yet to be achieved. A specific radioimmunoassay for human relaxin has yet to be established and presently published data on human serum relaxin levels are based on the porcine assay. Details of such a porcine relaxin radioimmunoassay utilised in these studies are described in Chapter 3.2.

1.6 DISTRIBUTION AND SITES OF SYNTHESIS

Relaxin has been found in the serum of all female mammals studied and in comparatively high quantities during pregnancy (Schwabe et al 1978). In the pig there are especially high levels in the corpus luteum of pregnancy and this has been a major source of relaxin. Relaxin has also been found in non-mammalian species (Steinetz, Beach & Kroc 1959), e.g. sharks, and has been isolated from the testes in the rooster and the armadillo. No relaxin has been found in human testes, but significant quantities have been found in human seminal plasma (Loumaye, Decooman & Thomas 1980); this has probably originated in the prostate (Kendall et al 1983).

The corpus luteum appears to be the main source of relaxin in women. Venous blood from the ovary containing the corpus luteum taken at Caesarean section contains several times as much relaxin as blood from the contralateral ovarian vein (Weiss, O'Byrne & Steinetz 1976). Luteectomy at Caesarean section leads to an abnormally rapid decline of the serum relaxin levels (Weiss et al 1977). Immunocytochemical techniques with antisera raised to a highly purified form of porcine relaxin (Kendall, Plopper & Bryant-Greenwood 1978) have localized relaxin to numerous small granules in the cytoplasm of the granulosa lutein cells in the pregnant sow. Recent detailed studies (Bigazzi 1981; Bryant-Greenwood 1981; Fields, Pardo & Larkin 1981) support older work by Zarrow & McClintock (1966) that human decidua is a source of active production. Recently there has been speculation that relaxin may be a neuropeptide as relaxin-like activity has been found in the pituitary gland.

1.7 SERUM LEVELS

In many species studied, relaxin levels rise during pregnancy and peak prior to parturition, suggesting a role for relaxin at that time. However, in the human, serum relaxin levels, as measured by heterologous RIA methods using porcine antisera, are highest in early pregnancy (Quagliarello et al 1979) reaching approximately 2 ng porcine equivalents/ml (Fig. 3). Thereafter, levels decline to approximately 1 ng porcine equivalents/ml during the second trimester and remain fairly steady at this concentration for the rest of pregnancy. When the expansion of plasma volume in pregnancy is taken into account, total relaxin production rates may be relatively stable throughout human pregnancy. However, studies by Bryant's group (Atele et al 1979) indicate that relaxin is secreted in a pulsatile manner and is rapidly cleared from the circulation and very frequent blood sampling must be carried out in order to obtain meaningful relaxin profiles. Most human studies have not achieved such a frequency of sampling prior to parturition, and therefore, a transient rise at this time, has not been excluded. Von Maillot et al (1977) claimed that tissue levels are highest at this time, suggesting that relaxin may have a role to play in human parturition. Postnatally, serum levels quickly drop to pre-pregnant levels (Quagliarello et al 1979); lactation or its suppression makes no difference to the rapidity of the decline. The sensitivity of porcine RIA is such that only a small fraction of the bioactivity of human relaxin may be measured. Also, since relaxin may act mainly as a 'local hormone', serum levels may not accurately reflect its biological role even when a human relaxin RIA is available.

Accepting these limitations, human relaxin levels as reflected in porcine equivalents using a porcine relaxin radioimmunoassay, have

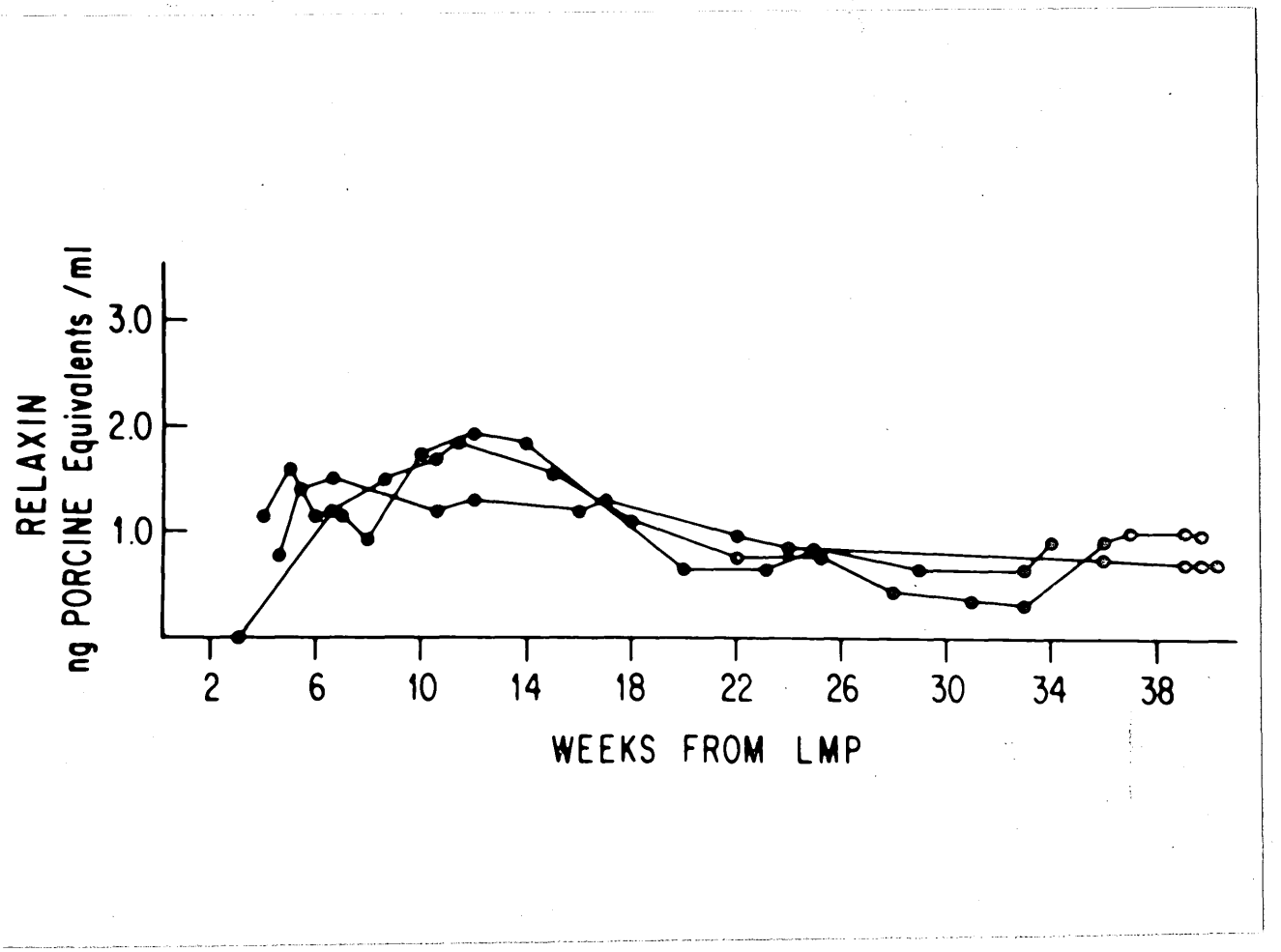


FIGURE 3 Serum relaxin levels during human pregnancy detected by a heterologous radioimmunoassay using porcine antisera. Reproduced with permission from Quagliarello et al (1979).

been reported in several obstetric pathologies (Szlachter et al 1982). Although relaxin levels were not higher during the third trimester of twin pregnancies they were significantly lower in post mature pregnancies and in patients who laboured prematurely. The authors speculate that lower relaxin levels in postmaturity might be associated with a delay in cervical ripening whilst lower relaxin levels in patients who laboured prematurely might decrease uterine quiescence and allow other factors to increase uterine activity. Evidence for relaxin's role in these areas in the human is discussed later in this thesis.

1.8 RECEPTOR SITES

The development of techniques to identify specific relaxin receptor sites is still in its infancy (Greenwood et al 1981). Specific binding sites fulfilling the rigid criteria of receptor sites have been described only in the rat uterus (Mercado, Bryant-Greenwood & Greenwood 1980). However, sites identified less rigorously have been reported in fibroblasts from mouse pubic symphysis and human skin (McMurty, Floershein & Bryant-Greenwood 1980) and in tissue from the cervix of the guinea-pig and the mammary gland of the rat (McMurty, Kwok & Bryant-Greenwood 1978).

In the rat, relaxin binding sites increase in direct relationship to the rise in plasma relaxin during pregnancy (Mercado et al 1980). However, at the time of the rat's prepartum surge in serum relaxin, an apparent reduction in relaxin receptor sites occurs. These sites may be under the control of oestrogen (Mercado et al 1980) or the oestrogen/progesterone ratio, although it is unlikely that such steroid levels are the only factors affecting the number or affinity of receptor sites. Lastly, direct correlation has yet to be made between specific relaxin receptor sites and biological response.

1.9 TARGET ORGANS AND BIOLOGICAL EFFECTS

Apparent physiological responses to relaxin have been found in many tissues.

1.9.1 Pubic Symphysis/Formation of the Pubic Ligaments

Relaxation of the pubic ligaments in response to relaxin after oestrogen priming was first described during pregnancy and parturition in the guinea-pig and later in mice. The changes in the connective tissue of the pubic ligaments involve dissociation of the collagen bundles, a net increase in total collagen, increased water uptake and a decrease in the viscosity of the ground substance which together lead to an increased flexibility and enlargement of the birth canal in these animals (Chihal & Epsey 1973). Pelvic ligament relaxation occurs to a much lesser degree in higher mammals and in human pregnancy changes in pelvic anatomy are comparatively minor, except in a few women who suffer from instability of their pelvic joints during pregnancy. No definite correlation of this clinical condition with relaxin has yet been published. However, data is presented in Chapter 6 suggesting there is a relationship between high peripheral serum relaxin levels and pelvic joint pain and laxity during human pregnancy.

1.9.2 Myometrium/Inhibition of Activity During Pregnancy

The tocolytic properties of relatively impure porcine relaxin on rat (Sawyer, Frieden & Martin 1953) and human (Kelly 1959) myometrial contractility in vitro were later confirmed in studies on rat and human non-pregnant myometrium using purified porcine relaxin (Porter, Downing & Bradshaw 1979; Szlachter et al

1980). Clinical studies in the 1950s suggested that crude relaxin in adequate dosage inhibited premature labour (Abramson & Reid 1955; McCarthy, Erving & Lauffe 1957; Eichner et al 1958). However, most of these studies were poorly designed and lacked adequate control groups. No clinical study using purified relaxin has been attempted to date. Relaxin's inhibitory effect on uterine contractions in vitro does not appear to be mediated through a beta-adrenergic mechanism (Porter, Downing & Bradshaw 1979). However, cyclic AMP concentrations are increased (Cheah & Sherwood 1980) and this is known to inhibit enzymes initiating smooth muscle contraction. Nevertheless, relaxin's mechanism of action at the myometrial cellular level has yet to be clarified.

The inhibitory effect of relaxin on rat, pig and human myometrial activity has not been comprehensively studied and such a study is described in Chapter 10 together with the synergistic role played by progesterone with relaxin during pregnancy. Relaxin's effect on uterine muscle activity may change during pregnancy possibly in response to other hormonal changes, e.g. the oestrogen/progesterone ratio. Progesterone, oestrogen and relaxin appear to play a synergistic role in the inhibition of myometrial activity in early pregnancy. However, at the end pregnancy under different hormonal conditions, relaxin's role may be to facilitate labour. In a recent randomized double blind clinical trial using purified relaxin by vaginal application (Chapter 11) one-third of patients near term laboured within 15 h, whilst there was no such effect in placebo groups. Whether the initiation of labour was indirectly due to relaxin's cervical ripening activity or whether relaxin has a direct effect on the myometrium is not clear but other studies described in this thesis suggest the former mechanism is more likely.

In non-pregnant rats, guinea-pigs and sheep, both relaxin and oestrogen inhibit spontaneous and prostaglandin-driven myometrial activity, but leave the uterus responsive to oxytocin (Porter 1979 a). It is possible, in some animals at least, that progesterone may inhibit premature labour indirectly by inhibiting the formation of cytoplasmic oestrogen receptor sites. A change in the oestrogen/progesterone ratio thus increases these receptor sites (Mercado et al 1980; Nissenson, Flouret & Hetchter 1978). Thus, withdrawal of progesterone is likely to enhance the oxytocin sensitivity of the myometrium and subsequent oxytocin-driven myometrial activity will not be inhibited by oestrogen or relaxin. A brief pre-labour surge and subsequent withdrawal of relaxin in some species may facilitate the precise timing of the onset of labour.

Very recent work by Koay et al (1983) suggests that relaxin may play a part in rupture of the fetal membranes. They cultured human amnion and chorion in vitro and found that added relaxin caused a corresponding increase in collagenase and plasminogen activator over a 32 h period. This work suggests that the fetal membranes may also be a target tissue for relaxin and that by stimulating the release of collagenolytic enzymes relaxin may contribute to the weakening and eventual rupture of the fetal membranes. This may be a gradual process as the clinical trials (Chapter 13) in Adelaide with exogenous porcine relaxin placed in the cervical canal and against the fetal membranes suggest that over a 15 h period such an application does not precipitate membrane rupture.

1.9.3 Uterine Connective Tissue/Pregnancy Accommodation

There is extensive uterine collagen synthesis during pregnancy and rapid degradation postpartum. Relaxin may play a major role in collagen biosynthesis and be responsible for both the proliferation of the uterine collagenous framework and the increase in uterine distensibility during pregnancy. Postnatally relaxin may directly or indirectly play a part in aiding uterine involution. Studies on combinations of relaxin, oestradiol and progesterone given to ovariectomized rats show that the greatest increase in uterine connective tissue occurs when all three hormones are given together (Cullen & Harkness 1964). In the rat, relaxin significantly augments the distensibility of the uterus in pregnancy (Wiqvist 1959) and has protein and carbohydrate anabolic effects on the uterus (Vasilenko, Frieden & Adams 1980). Histological studies (Chapter 14) in our laboratories have shown that in late pregnancy pure porcine relaxin applied vaginally is associated in the rabbit with an apparent dissolution on the cervical stromal connective tissue 18 h after application. The role of relaxin in uterine accommodation during human pregnancy awaits study.

1.9.4 Endometrium/Preparation for Implantation

In 1967, Hisaw et al showed that the development of full histological changes of the endometrium in early pregnancy in the rhesus monkey depended on the presence of the three hormones - oestrogen, progesterone and relaxin. Relaxin appears to augment the progestational changes. Large doses of oestrogen inhibit relaxin's effect. Subcutaneous injections of relaxin in the non-pregnant monkey also induce hyperplasia of the endothelium of maternal endometrial blood vessels (Hisaw et al 1967). Such

endothelial changes are normally seen in early pregnancy. These effects suggest a possible role for relaxin in the preparation of the endometrium for implantation of the blastocyst.

A continuous infusion of relaxin during rat blastocyst implantation interferes with normal blastocyst spacing (Chapter 8). In these experiments we found that all implantation sites were confined to the upper half of each horn instead of being evenly spaced along the lumen and the orientation of the embryonic discs were also disrupted. Thus, exogenous relaxin appeared to inhibit normal distribution of the blastocysts possibly through decreasing uterine muscle contractility or by a direct effect on the endometrium. Resulting crowding of the blastocysts may have been responsible for the atypical implantation sites around the uterine lumen. Studies on the role of relaxin in human implantation are described in Chapter 9.

1.9.5 Cervix/Cervical Ripening

In most species, including the human, the cervix undergoes marked changes in its structure prior to parturition. A softening, shortening and opening of the cervix is associated with histological changes in the cervical stroma as mentioned below. Although the precise mechanism of cervical ripening and the initiation of parturition is not known several hormones are known to influence these processes (Fig. 4).

There have been many studies in which relaxins of differing purity have been used showing that relaxin causes cervical ripening in a variety of animals (Zarrow et al 1956; Stone, Sedlis & Zuckerman 1959; Hollingsworth, Isherwood & Foster 1979; Kertiles & Anderson 1979). Some conflict in the results of these early studies was probably due to the impurity of the preparations

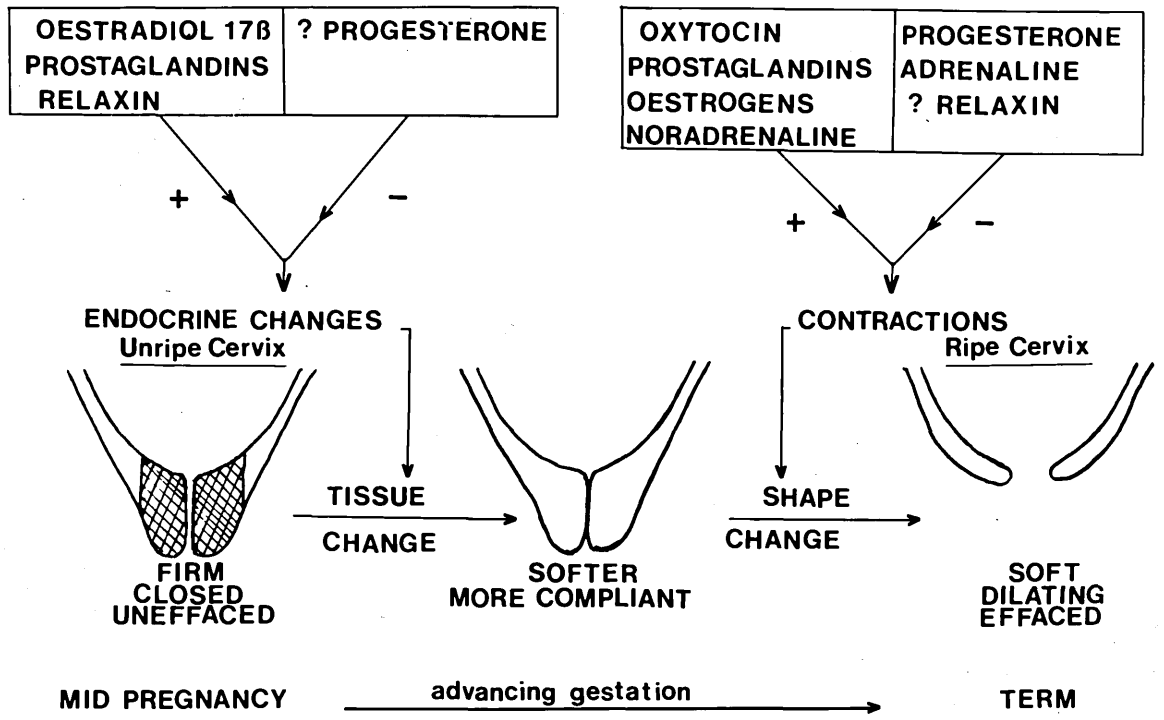


FIGURE 4 The hormones in the human which may promote or inhibit cervical ripening and uterine contractility.

used. However, a recent double blind randomized controlled trial in the human using purified porcine relaxin showed that vaginal application of relaxin to patients near term was associated with cervical ripening in 85% of patients so treated (Chapter 11). Animal studies (Steinetz, O'Byrne & Kroc 1980) suggest that relaxin-induced cervical ripening is similar to that seen before parturition and that the connective tissue changes are similar to those seen in the pubic symphysis and uterus. In these connective tissues, the collagen fibres become loose and widely separated, whilst the viscosity of the ground substance decreases. A major increase in glycosaminoglycans, together with a slight increase in the water content of the cervix, contribute to a large increase in the volume of the ground substance. A rise in total cervical collagen also occurs, but the increase in ground substance is greater, leading to a relative dilution of the collagen within the cervical tissue (Steinetz et al 1980).

Evans et al (1983) have confirmed the cervical ripening effect of porcine relaxin near term by giving 2 mg porcine relaxin pessaries in the cervical canal. In their trial these relaxin treated patients had shorter induction/delivery intervals compared to the control group. This is in keeping with the clinical trial of MacLennan et al (1980) which used 2 mg of porcine relaxin in a vaginal gel 15 h prior to surgical induction of labour. In this latter trial the relaxin treated group had significantly shorter labours and less need for obstetric intervention during labour and delivery. In a further trial (Chapter 12) combinations of relaxin with other cervical ripening agents, i.e. oestradiol and prostaglandin $F_2\alpha$ did not enhance their individual effects suggesting that these hormones may act in sequence to produce the same cervical ripening effect rather than

to act in concert to produce complementary effects.

1.9.6 Mammary Glands: Lactation

Early studies suggested that crude relaxin extracts promoted mammary gland connective tissue growth in rodents and that relaxin was synergistic with other steroid hormones in promoting breast tissue growth during pregnancy (Schwabe et al 1978). Relaxin binding sites have been described in rat mammary tissue (Zarrow & McClintock 1966; McMurty et al 1978). In the pig, plasma relaxin is increased by suckling (Atele et al 1979), but lactation in the sow can also be inhibited or reduced by injections of exogenous relaxin towards the end of pregnancy (Steinetz et al 1980). Although serum relaxin levels in the human rapidly delcine after parturition (Quagliarello et al 1979), detailed studies during suckling have not been carried out. Relaxin's role, if any, in human breast growth and lactation has not been established.

1.9.7 Semen/Sperm Motility

Relaxin is present in human seminal plasma (Loumaye, Decooman & Thomas 1980). When purified porcine relaxin is added to washed human sperm from both semen samples with normal sperm motility and from subfertile specimens with reduced motility, relaxin attenuated the normal decline in sperm motility and forward progression of the sperm (Essig et al 1982). Further support to a role for relaxin in enhancing sperm motility has been reported by Sarosi et al (1983), who found that anti-porcine relaxin antibody quickly and significantly impaired human sperm motility even when diluted to very low concentrations. They suggest a potential for anti-relaxin antibody as an adjuvant in

barrier contraception and also for such sperm immobilization to be used as a rapid screening procedure for anti-human relaxin antisera.

The exact role of relaxin in human seminal plasma has yet to be defined but clearly it is another factor that facilitates sperm motility. Other possible roles for seminal relaxin that require investigation are cervical mucus penetration, relaxation of the uterotubal sphincter and ovum penetration.

1.9.8 Skin: Collagen Disorders

The possibility that relaxin may exert an effect outside the reproductive tract has barely been explored. Certainly there are minor changes in skin connective tissue during pregnancy. McMurtry et al (1980) reported specific relaxin binding sites on fibroblasts from human skin. Their data also suggest that relaxin is mitogenic to skin fibroblasts in vitro. However, the binding of purified relaxin to skin fibroblasts or to any other binding sites has yet to be shown to have any biological consequence. Casten & Boucek (1958) reported an increase in the elasticity of the skin in patients with scleroderma when treated with crude extracts of relaxin. Similar clinical studies have not yet been reported with purified relaxin. A specific role for relaxin in non-reproductive disease, in particular the collagen disorders and arthritis, warrants further investigation.

1.10 MECHANISM OF ACTION

Relaxin appears to influence collagen metabolism in most target tissues. In vitro studies on the rat pubic symphysis suggest that relaxin activates the collagenolytic system by raising tissue levels of collagenase and collagen peptidase, an enzyme involved in the degradation of denatured collagen (Weib, Magelschmidt & Struck 1979). The mitogenic action of relaxin, on the other hand, appears to stimulate fibroblast replication in the target tissue and concomitantly, the synthesis of new collagen. Thus, relaxin appears to stimulate collagen biosynthesis by increasing both collagen production and breakdown to allow remodelling of the target tissue, thereby giving it greater distensibility and pliability to accommodate the growth of the pregnancy. Cyclic AMP may serve as a second messenger in the target tissues to mediate other biological responses - e.g. the inhibition of uterine activity - since cyclic AMP levels are significantly elevated after relaxin administration in the uterine and cervical tissues (Cheah & Sherwood 1980).

The net effect of relaxin's action on protein and carbohydrate metabolism is anabolic (Vasilenko, Frieden & Adams 1980), collagen synthesis being greater than collagen degradation with a resultant increase in the total collagen content of the target tissues. Relaxin also effects a decrease in the viscosity of the intercellular ground substance (Zarrow et al 1956; Leppi & Kinnison 1971) by increasing its water and glycosaminoglycan content. The resultant increase in volume of the ground substance is greater than the net increase in collagen and thus there is a relative decrease in the collagen concentration of the target tissues allowing increased extensibility and remodelling (Fig. 5). The evidence for the mechanisms of action of relaxin has mostly been derived from animal studies and clear evidence for such a role in the human remains to be presented.

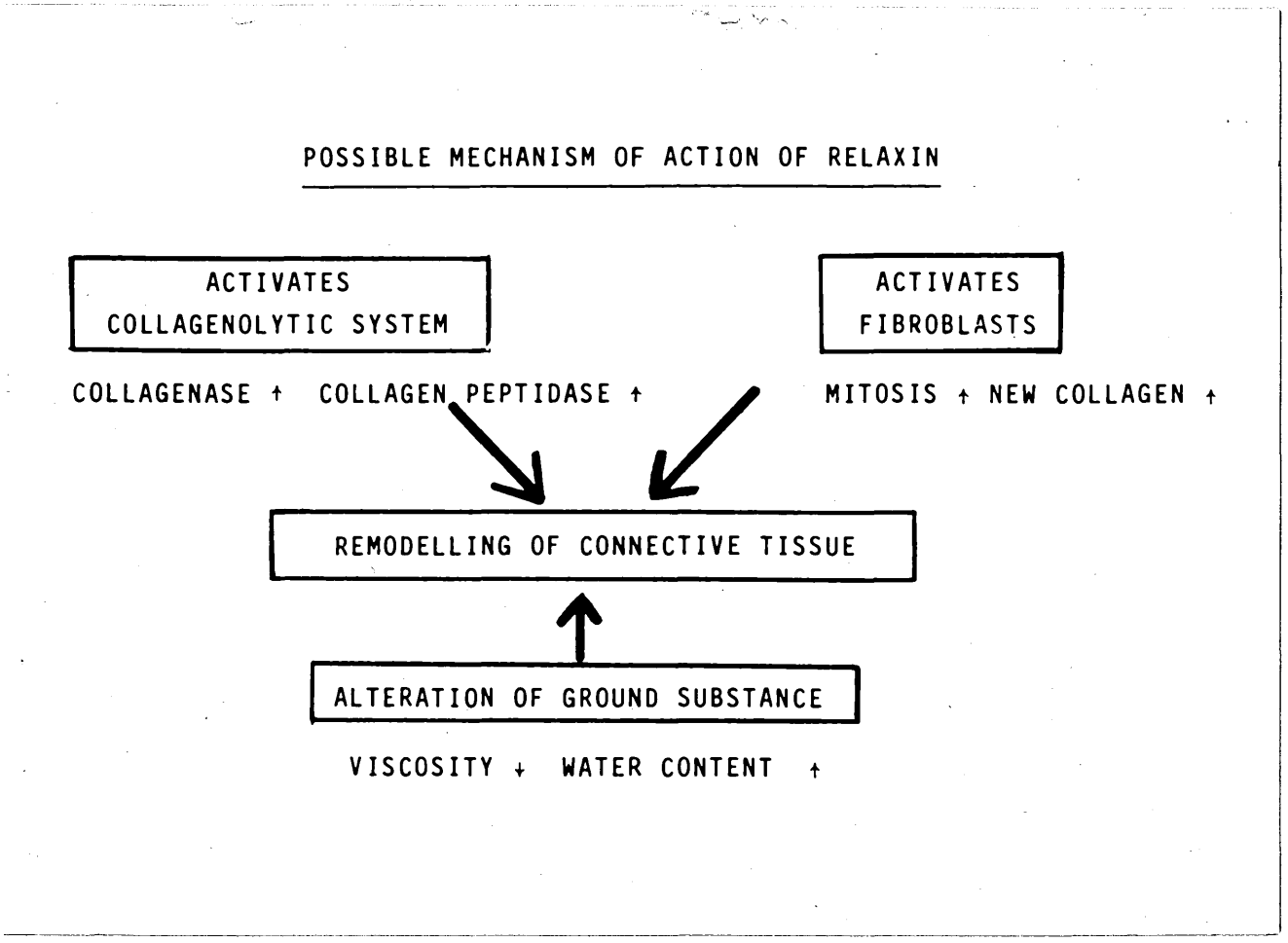


FIGURE 5 Possible mechanism of action of relaxin in target tissues.

1.11 CONTROLLING MECHANISMS

Oestrogen priming is required before relaxin can exert its effect on the guinea-pig pubic symphysis (Hisaw 1926; Fevold et al 1930) and inhibition of steroid synthesis in the pregnant rat inhibits relaxin's effect of cervical softening (Schwabe et al 1978). Oestrogen and progesterone are required for relaxin to exert its effect on the endometrium (Hisaw et al 1967). In some animals, oestrogens may effect the release of relaxin (Porter 1979b) and recent work suggests that in the rat, oestrogen may also stimulate an increase in relaxin receptor sites (Mercado et al 1980). Thus, there is a close but not precisely defined relationship between oestrogen and relaxin and, as discussed earlier, it is possible that differing ratios of oestrogen and progesterone may mediate in the changing of relaxin's role in pregnancy.

Some aspects of the relationships of other pregnancy hormones to relaxin have been studied. Whereas the prolactin level does not affect relaxin levels in the rat (Schwabe et al 1978) prostaglandins do influence the action of relaxin in this and other animals. Prostaglandin synthetase inhibitors block the cervical ripening effect of relaxin in rats, suggesting that this action may be mediated by prostaglandins (Kennedy 1976). Indeed, injection of $PGF_2\alpha$ in the pregnant sow induces a rapid increase in relaxin levels (Sherwood et al 1976), and recent studies on the rat suggest that $PGF_2\alpha$ causes cervical ripening indirectly by acting on the ovary to release relaxin. However, in a clinical study of seven patients given intravenous $PGF_2\alpha$ a rise in serum level of relaxin was not detected (Hochman et al 1978). $PGF_2\alpha$ and relaxin, separately, produced similar cervical ripening effects in the human when administered vaginally at term (MacLennan et al 1980; MacLennan & Green 1980), but no significant additive, synergistic or inhibitory effect occurred when they were

given in combination (MacLennan 1981).

Lastly, with regard to controlling mechanisms, it is probable that the corpus luteum of pregnancy secretes relaxin in response to a fetal-placental stimulus soon after conception. Human chorionic gonadotrophin, prolactin and placental lactogen apparently fail to stimulate increased relaxin formation in incubations of human term corpora lutea (Schwabe et al 1978). However, injections of HCG in non-pregnant women will stimulate relaxin production if injected from 8-10 days after ovulation (Quagliarello et al 1980). No detectable serum levels of relaxin are found when HCG injections are given 2-3 days after ovulation. Thus, the age of the corpus luteum is an important determinant in the relaxin response to HCG and HCG may be the stimulus to relaxin production in human pregnancy.

1.12 SPECULATION ON THE ROLES OF RELAXIN IN THE HUMAN

The first human clinical trials using purified porcine relaxin are described in this thesis and examine relaxin's role in facilitating implantation, cervical ripening and in initiating labour near term. This latter effect may be in response to its cervical ripening action.

Other roles for relaxin in the human are more speculative, the evidence being indirect and derived from animal models, in vitro studies of human tissues and from heterologous RIA methods as described in Chapters 5, 6 and 7. This indirect evidence suggests that during pregnancy relaxin alters collagen biosynthesis in the reproductive tract to facilitate remodelling of these tissues. Specifically, relaxin may play an important part in (i) implantation, facilitating invasion of the decidual connective tissue by the developing trophoblast, (ii) uterine growth, allowing accommodation of the growing pregnancy by promoting remodelling of the uterine connective tissue and (iii) in the inhibition of myometrial activity until late pregnancy. Direct evidence of these postulated roles in the human is needed. Relaxin may have other roles related to its effect on collagen metabolism in human reproduction, as there is at least suggestive evidence that it may facilitate rupture of the follicle at ovulation (Chihal & Epsey 1973), enhance sperm penetration (Essig et al 1982) and, like its structural relatives, insulin and nerve growth factor, it could possibly be a fetal growth factor (Hall & Sara 1978). Relaxin's relatively established and more speculative roles in mammals are tabultaed in Table 1. Lastly, relaxin may not be solely a hormone of pregnancy, as it has been detected in non-pregnant women, and men. A role for relaxin that is not pregnancy-related has not been identified, but it could play a part in the control of connective tissue dynamics.

ESTABLISHED IN SOME SPECIES
1. Relaxation of pelvic ligaments near parturition
2. Uterine stromal remodelling during pregnancy
3. Inhibition of myometrial activity during pregnancy
4. Cervical ripening and facilitation of parturition
5. Mammary growth during pregnancy
6. Enhancement of sperm motility
SPECULATIVE
1. Facilitation of follicle rupture at ovulation
2. Facilitation of blastocyst implantation
3. Fetal growth factor
4. Facilitation of fetal membrane rupture
5. Factor influencing skin collagen metabolism and collagen disorders

TABLE 1 Possible biological roles of relaxin in mammals

This thesis describes some of the candidate's ongoing studies on the role of relaxin in human reproduction and specifically describes relaxin levels in normal and abnormal human pregnancy in vitro, studies on the effect of relaxin on myometrial activity and the first clinical trials with pure porcine relaxin in the areas of human embryo implantation, cervical ripening at term and the induction of labour.

CHAPTER 2

THE PURIFICATION OF PORCINE RELAXIN

2.1 INTRODUCTION

In each of the studies described in this thesis porcine relaxin was purified by the methods described in this chapter. The methodology is based on extraction process of Sherwood and O'Byrne (1974). This process finally gives three bioactive relaxin peaks (CMB, CMa and CMa') on elution from a carboxymethyl cellulose column (Fig. 1 Chapter 1). The complete porcine relaxin polypeptide contains 31 amino acids in its B chain and this form is the main constituent of the CMa and CMa' peaks. The contiguous peaks are due to molecular variants with shorter B chains, e.g. B29 relaxin and B30 are artefacts of the isolation procedure due to proteolysis occuring during purification (Walsh and Niall, 1980).

To minimise proteolysis and obtain a single peak of B31 relaxin the method of Sherwood and O'Byrne has been adapted according to the method of Walsh and Niall (1980) (with minor modifications) where octadecylsilica columns are used in the elution process. All steps in the purification process described below were performed in a cold room (4^{o}C) to minimise proteolysis.

2.2 COLLECTION OF OVARIAN TISSUE

Pregnant sow ovaries were snap frozen in liquid nitrogen within two minutes of slaughter at the South Australian Metropolitan Abattoir. The ovaries used were only collected from pigs whose carcases had been certified by Commonwealth Health inspectors as being appropriate for human consumption. The ovarian tissue was stored at -80°C until further processing.

2.3 PURIFICATION PROCESS

This process is summarised in Figure 6.

(1) Approximately 1 Kg of frozen pregnant sow ovaries were used in each purification process having been cut into 20G pieces. These pieces were individually homogenised for two minutes using a Dorvall Orini Mixer Homogeniser.

(2) Five ml of extraction buffer (0°C) per gram of tissue was added to the semi frozen homogenate.

Extraction buffer : 15% trifluroacetic acid (v/v)
5% formic acid (v/v)
1% sodium chloride (w/v)
1M hydrochloric acid

(3) The homogenate was then centrifuged at 2,600 r.p.m. at 4°C for 30 minutes and the precipitate discarded.

(4) The supernatant was then filtered through Whatman filter paper (No. 541) and thereafter through a 0.45 μ millipore filter.

(5) Three small disposable octadecylsilica (ODS,618) columns (Sep-Paks, Waters Associates) were connected in series and equilibrated by pumping 30 ml of an 80% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid solution through them using a Master-flex peristaltic pump at 5 ml/minute. The system was then washed through in a similar manner with 30 ml distilled water.

The effluent from the octadecylsilica columns was collected and pumped through the columns for a second time. During this process the columns became yellow-brown in colour from absorbed protein. They were then washed with 30 ml of a 10% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid solution and the effluent discarded.

(6) Relaxin and other ovarian peptides were then eluted from the columns with 40 ml of the 80% acetonitrile, 0.1% trifluoracetic acid

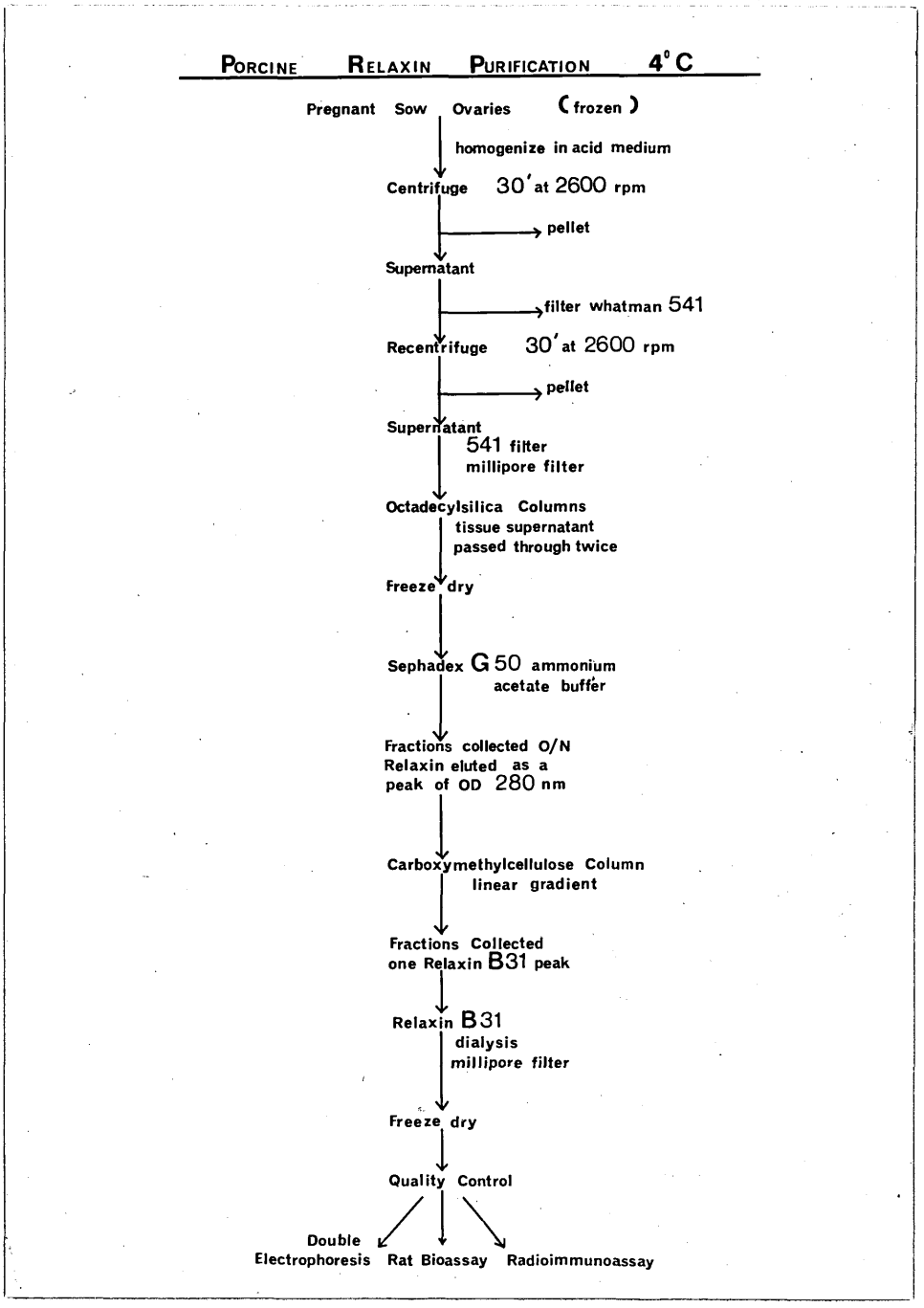


FIGURE 6 Summary of the biochemical steps in the purification of porcine relaxin.

solution. During this process the yellow-brown discolouration is removed from the columns.

(7) The eluant was lypholysed to near dryness by rotary film evaporation at 38°C.

(8) The lypholysed compound was reconstituted with 1-2 ml 0.01M ammonium acetate, pH 5.0, and applied to a 1.5 x 100 cm column of G5D Superfine Sephadex equilibrated with the same buffer.

(9) The fractions were collected overnight and the relaxin (mol. wt. 6,300) is eluted as a peak at optical density 280 nM (Fig. 7 - Peak A). The column was developed at a rate of 0.5 ml/minute using a peristaltic pump and 1 ml fractions were collected. The relaxin activity as measured by bioassay (Chapter 3) and by radioimmunoassay (Chapter 4) elutes as a symmetrical peak of optical density (280 nM) at a position corresponding to a molecular weight of approximately 6,000 daltons.

(10) The eluted peak was pooled and pumped directly onto a 10 mm x 10 cm carboxymethylcellulose column equilibrated with 0.01M ammonium acetate, pH 5.0, conductivity 0.6 mS. This column was developed with a linear salt gradient of ammonium acetate, pH 5.0, to a final conductivity 15 mS, at a flow rate of 0.5 ml/minute.

(11) The purified relaxin eluted, at about 14 mS, conductivity, as a single peak (Fig. 8 - Peak B) which was pooled and dialysed against double distilled water.

(12) The resulting pure porcine relaxin was then lypholysed as in step 7 and stored at -80°C.

Using this method approximately 90-100 mg of pure porcine relaxin was produced from 1 Kg of mid to late pregnant sow ovaries and approximately 500 mg of pure porcine relaxin was produced for the in vitro, animal and clinical trials described in this thesis. All batches of relaxin were subjected to the quality control procedures described in Chapter 2 (2.4).

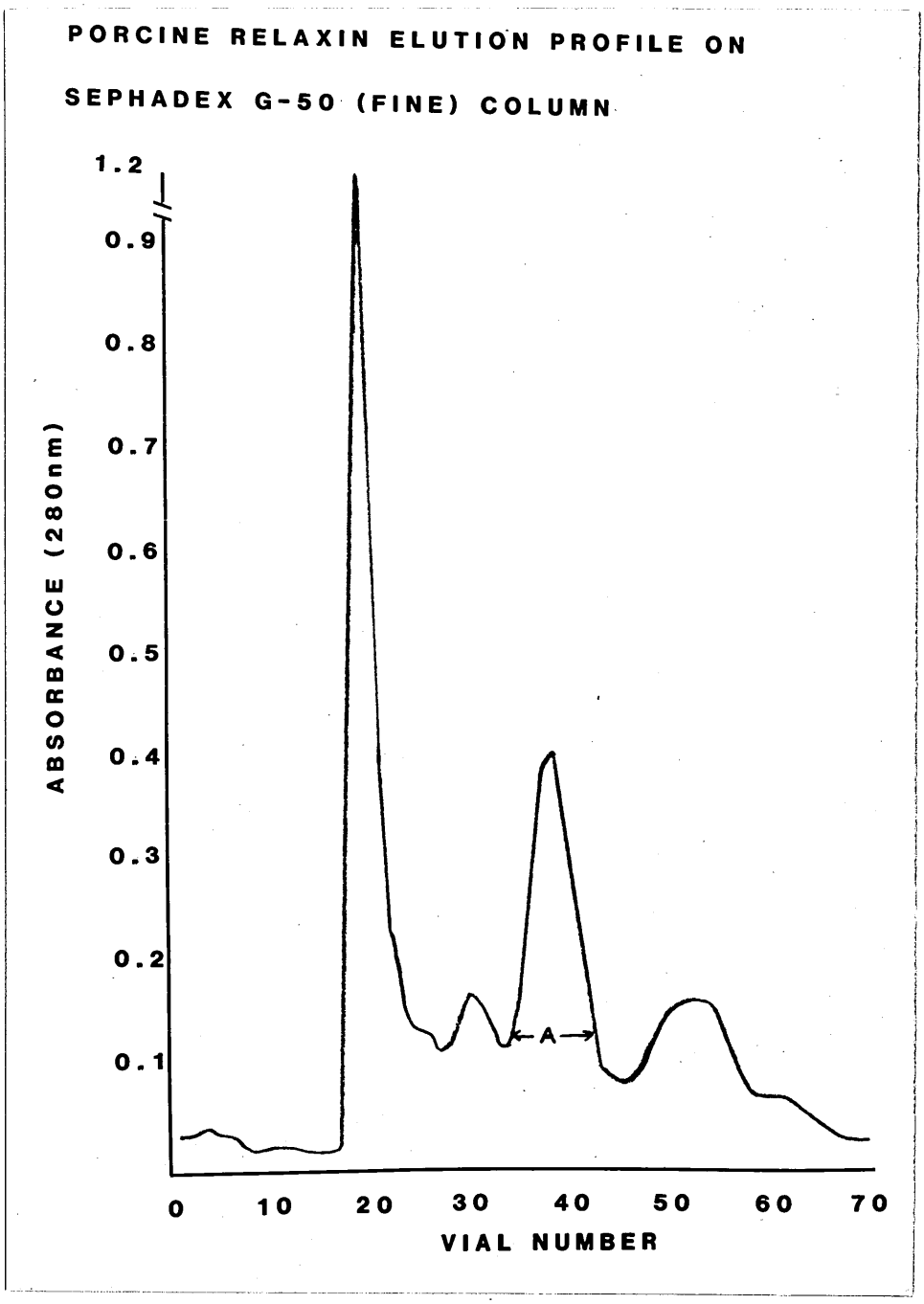


FIGURE 7 Porcine relaxin elutes as peak A on a Sephadex G50 column.

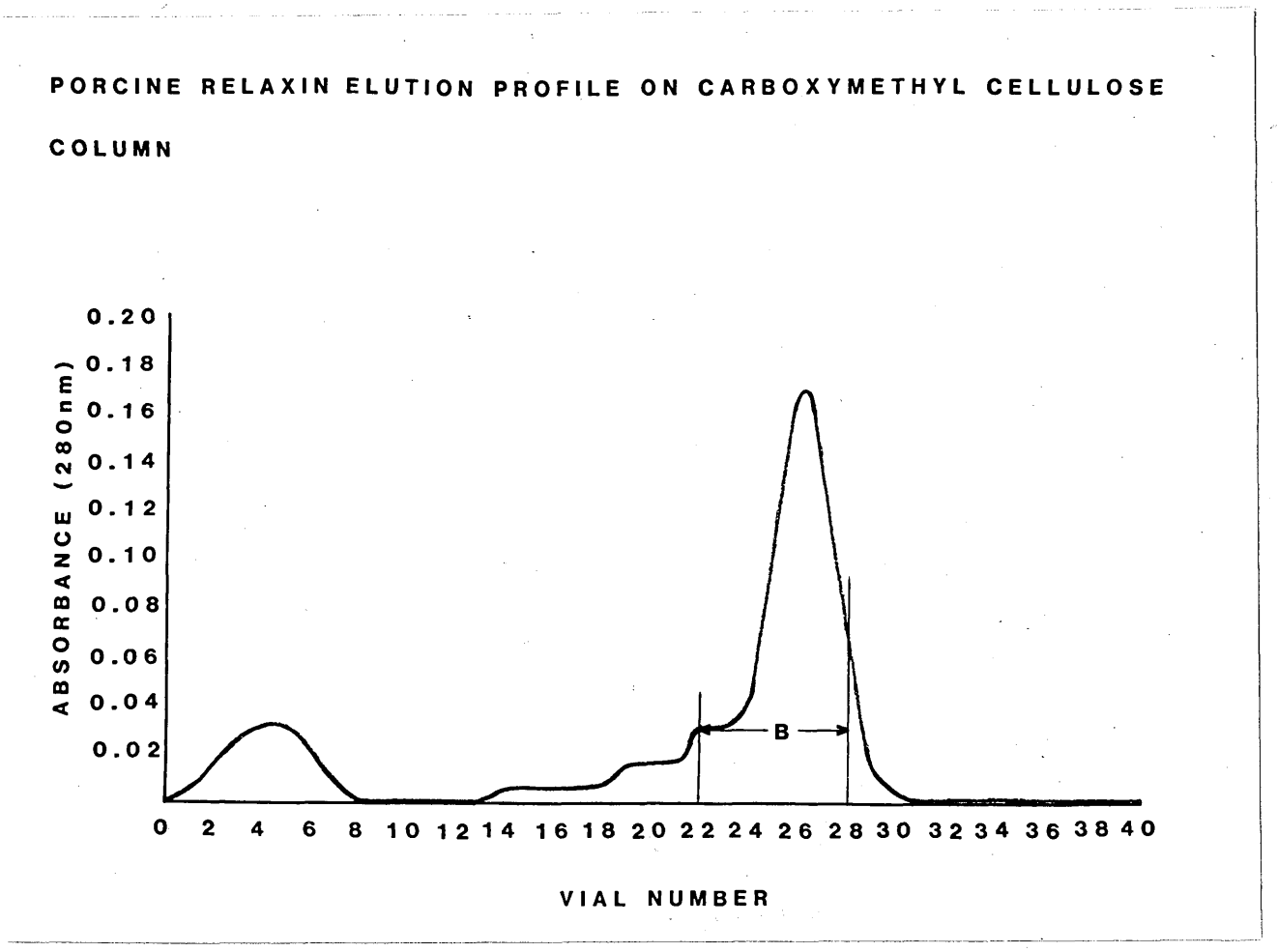


FIGURE 8 Porcine relaxin elutes as a single peak (B) on a carboxymethyl cellulose column.

2.4 QUALITY CONTROL OF PORCINE RELAXIN PURIFICATION

2.4.1 Bioassay

At the end of step 9 in the previously described methodology for the purification of porcine relaxin and after the final step the product was tested for relaxin activity using the rat myometrial activity inhibition bioassay and quantitatively in the rat myometrial bioassay of St. Louis (1981) as described in Chapter 3. The biopotency was compared to the reference preparation (NIH-R-PI) and to the reference preparation (CMa) used for the analysis of the primary structure and sequence studies of porcine relaxin (James et al 1977) which was kindly supplied by Prof. F.C. Greenwood, University of Hawaii. All batches used in the studies were of comparable biopotency to the reference preparations (Fig. 9).

2.4.2 Radioimmunoassay

Each batch was assayed by radioimmunoassay as described in Chapter 4. The activity of the porcine relaxin produced in our laboratories closely matched that of the reference preparations (Fig. 10).

2.4.3 Double Electrophoresis

Each batch was also subjected to double electrophoresis to further test for contaminants (Fig. 11). No batch showed evidence of negatively or positively charged contaminants, a single spot remaining on the solvent front after double electrophoresis.

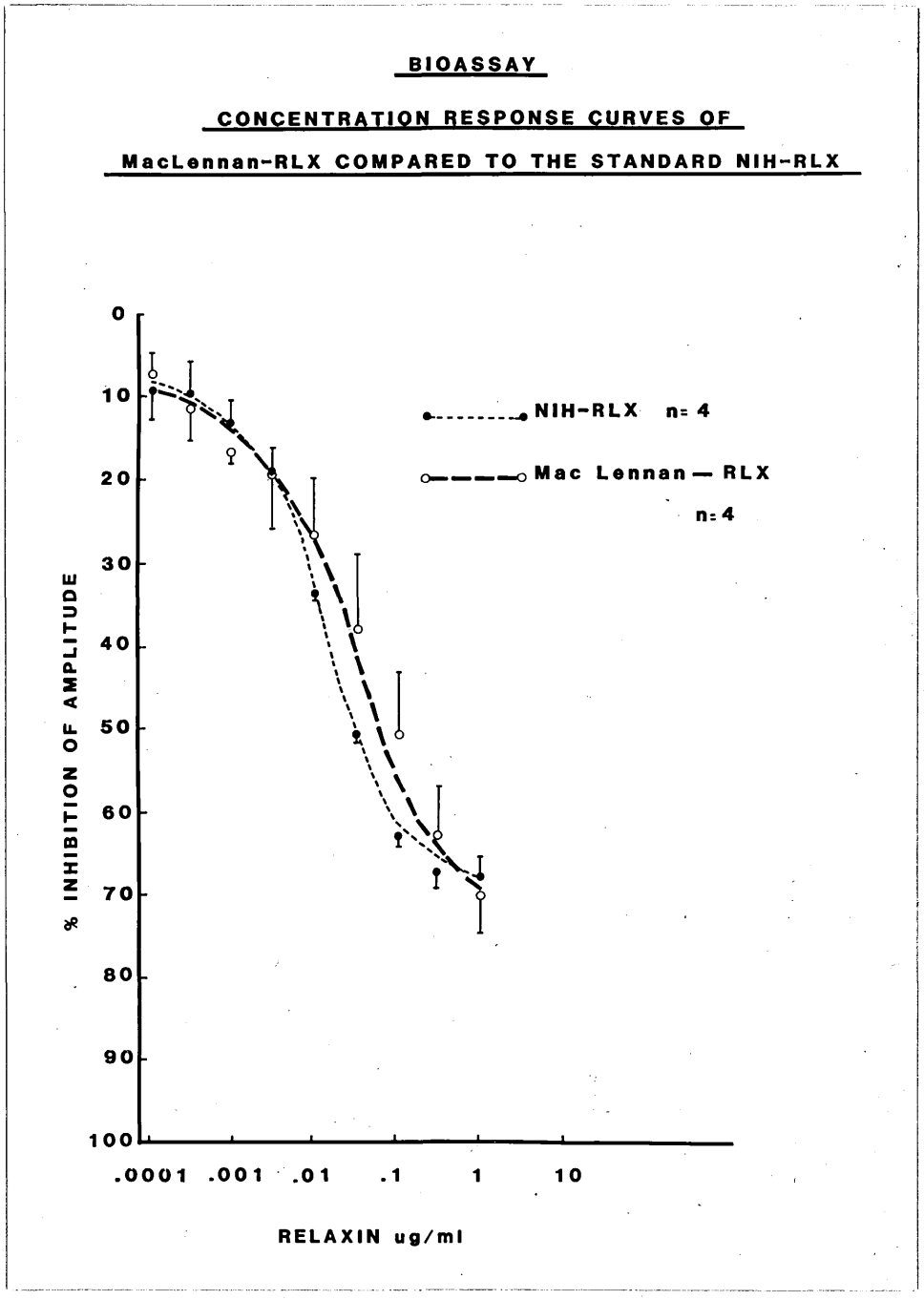


FIGURE 9 Comparison of the concentration response curves of porcine relaxin purified for these studies compared to the NIH standard porcine relaxin when measured by the bioassay of St. Louis (1981).

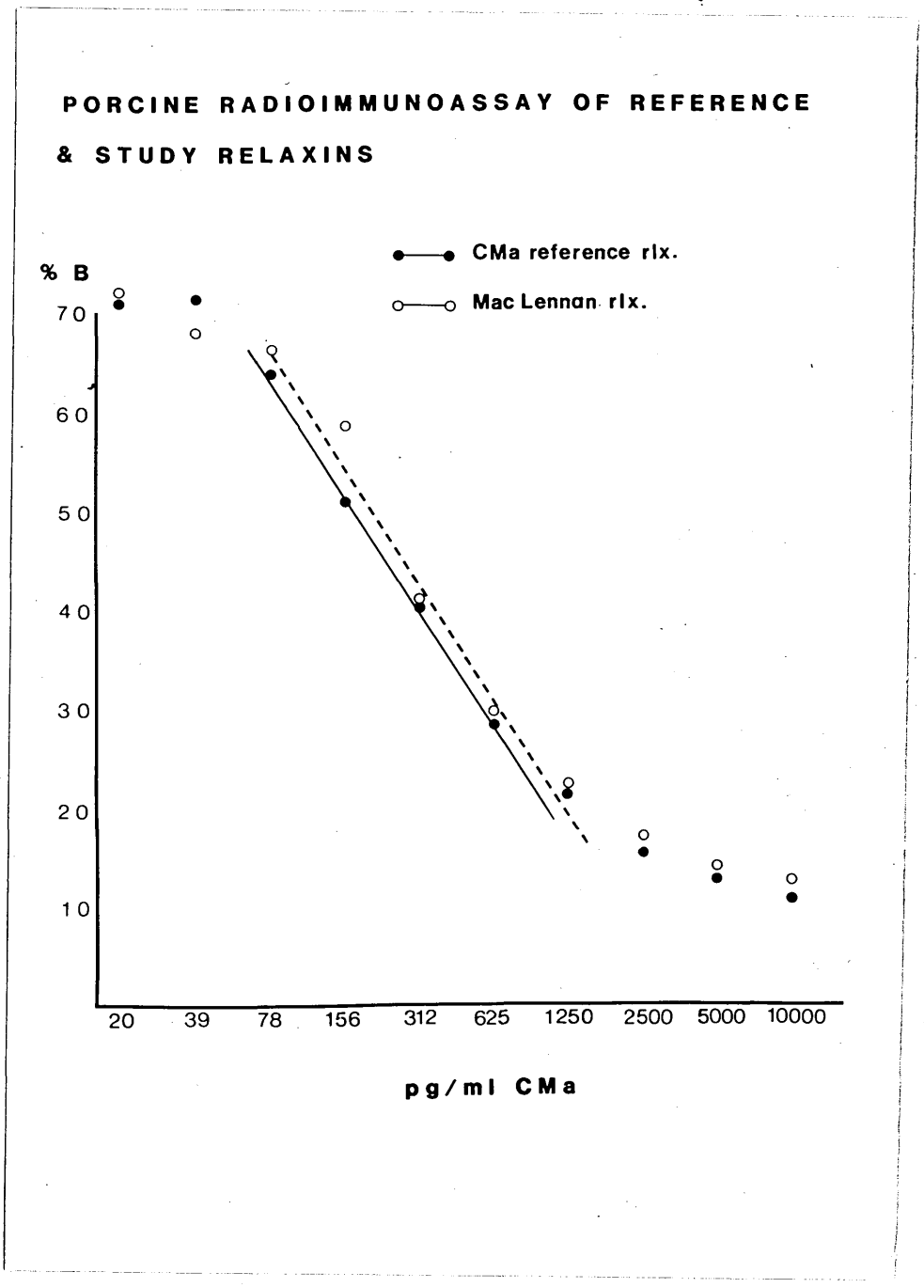


FIGURE 10 Comparison of the concentration response curves of porcine relaxin purified for these studies compared to the NIH standard porcine relaxin (CMa reference rlx.) when measured by porcine relaxin radioimmunoassay (% B = percentage binding).

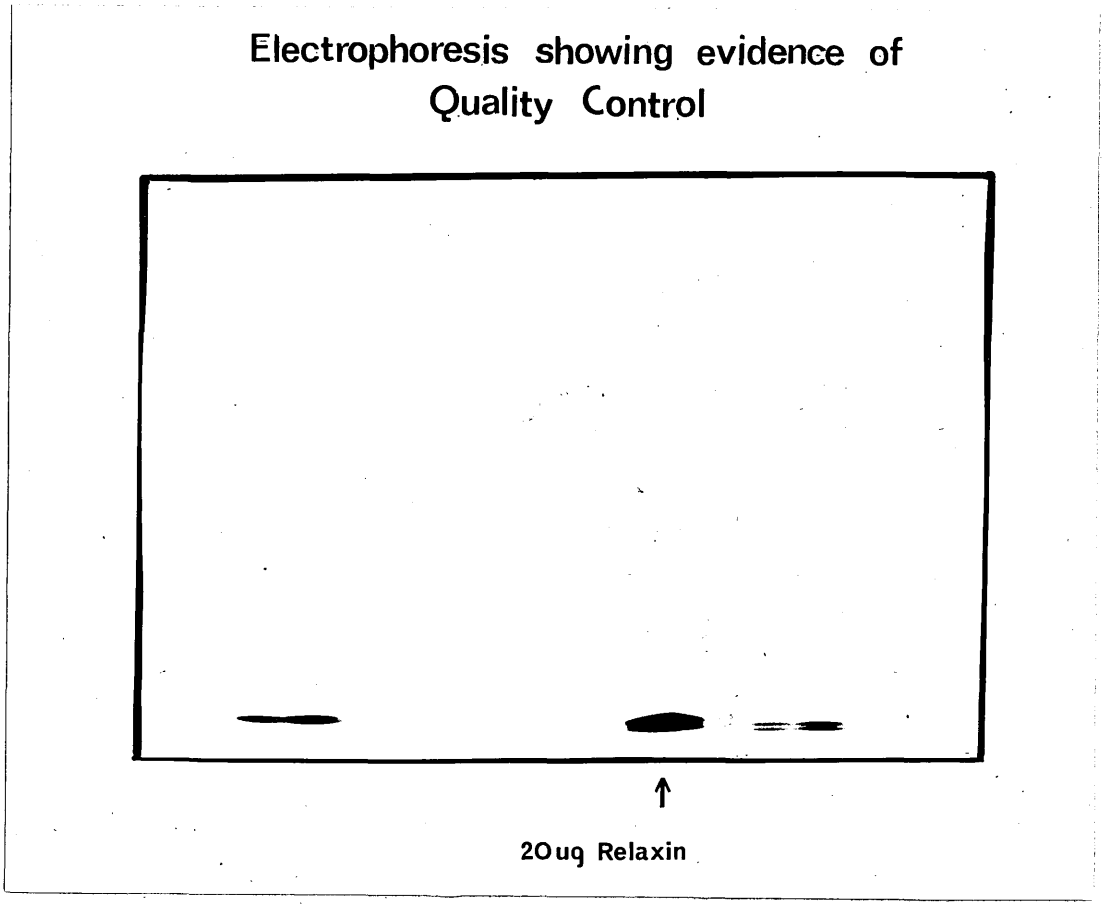


FIGURE 11 Double electrophoresis of porcine relaxin purified for these studies showing a single spot remaining on the solvent front and a lack of negatively or positively charged contaminants.

2.4.4 Bacteriological Testing

The relaxin used in the clinical trials was prepared by the hospital pharmacist aseptically in a sterile laminar air flow area by diluting the required amounts (1 or 2 mg) of relaxin in sterile water. This was subjected to millipore filtering and the dissolved relaxin was stored at $4^{o}C$ in sterile vials. These were subjected to random sampling for bacterial analysis following the pharmaceutical protocols of the Australian National Biological Standards Committee. No batches showed any sign of bacterial contamination.

CHAPTER 3

THE BIOASSAY OF PORCINE RELAXIN

3.1 INTRODUCTION

Two assays were used. The first was used as a qualitative test for the presence of relaxin-like activity. However, because the spontaneous activity of myometrium declines with time the second assay is a better and more reproducible quantitative assay.

3.2 THE INHIBITION OF SPONTANEOUS ACTIVITY IN RAT MYOMETRIUM

This assay involves the inhibiton of spontaneous uterine motility *in vitro* in specimens taken from oestrogen-primed rats and is based on the method of Wiqvist and Paul (1958) with minor modifications. Virgin rats (200-250G) were primed with a single injection of 0.2 mg oestradiol valerate 24 h prior to decapitation. The uterine horns were removed and cut into 0.5-1.0 cm segments. Each segment was suspended in Earle's solution by a cotton thread in a 15 ml double-jacketed chamber. The solution was corrected to a physiological pH. Temperature was maintained at 37°C and there was a constant flow of 95% oxygen and 5% carbon dioxide. Isometric contraction of each uterine segment was recorded on a Gibson polygraph calibrated to a tension of 1G. After the spontaneous contraction of the uterine segment had been established the presence of relaxin bioactivity could be detected by the addition of the unknown sample in varying concentrations to the Earle's solution in the chamber and the subsequent inhibition or diminution in the frequency and amplitude of the contractions (Fig. 12). Each sample was run in triplicate and the return of myometrial activity confirmed after washing of the muscle with Earle's solution. Comparison of the effects of known relaxin standards could be performed but more precise quantitation of effect was found with the assay described below.

INHIBITION OF SPONTANEOUS RAT MYOMETRIAL CONTRACTILITY

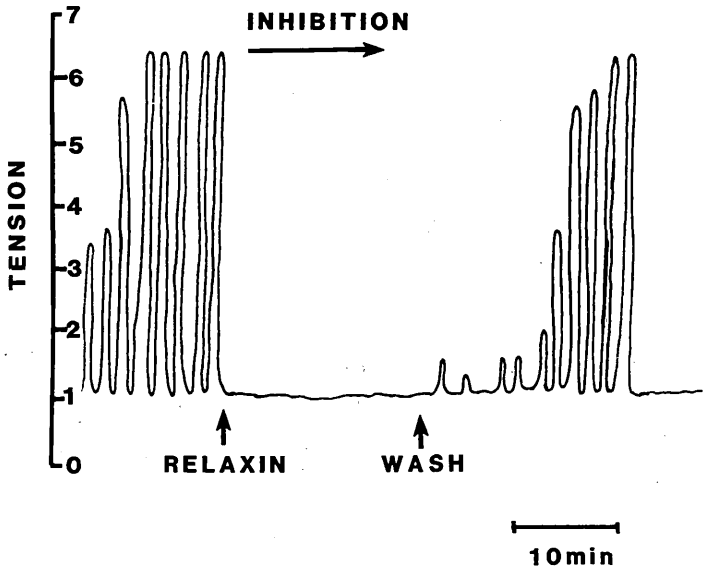


FIGURE 12 Bioassay for the presence of relaxin-like activity involving the inhibition of spontaneous myometrial activity in the oestrogen-primed non-pregnant rat.

3.3 THE INHIBITION OF KCl-INDUCED CONTRACTILITY IN RAT MYOMETRIUM

This is the bioassay described by St. Louis (1981). It also uses oestrogen-primed rat myometrium set up in a similar manner to the description in the previous bioassay. However, the chamber contains a 20% KCl solution in physiological saline. This induces a tetanic contraction of the myometrium giving a stable plateau on the polygraph recording (Fig. 13). The dose response effect of a relaxant can be measured as a percentage of the reduction in the height of the plateau and these responses can be titrated against known standard concentrations of pure relaxin.

INHIBITION OF KCL-INDUCED RAT MYOMETRIAL CONTRACTILITY

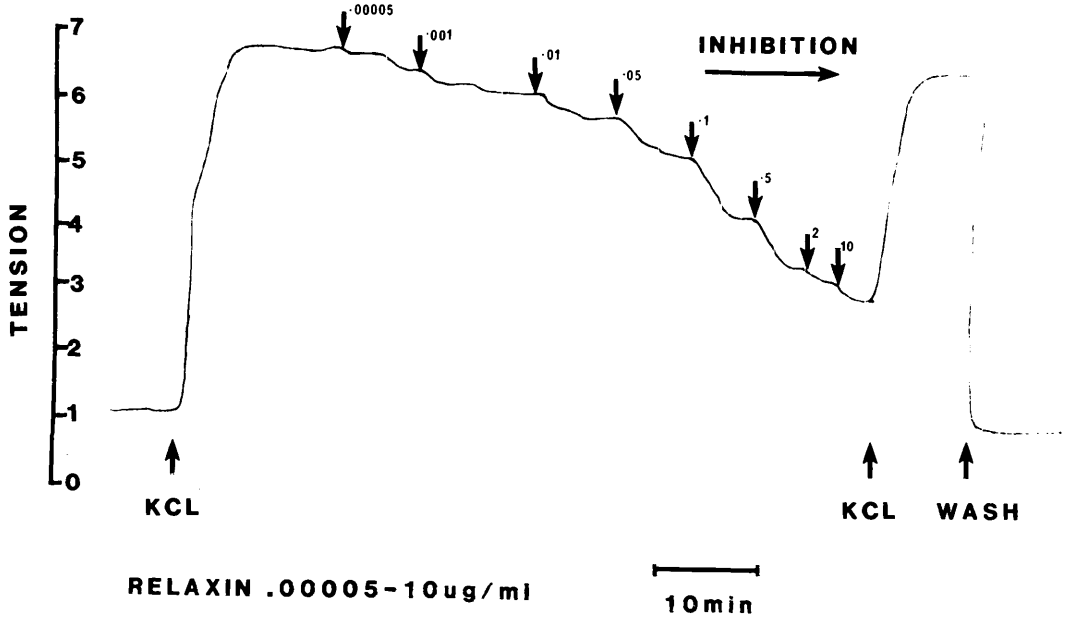


FIGURE 13 Bioassay of St. Louis (1981) for relaxin-like activity involving the inhibition of a KCl-induced tetanic contraction of oestrogen-primed rat myometria. The arrows pointing downwards represent the addition of increasing concentrations of the relaxin being studied, which quantitatively decreases the amplitude of the contraction.

4.1 INTRODUCTION

As yet no homologous human relaxin radioimmunoassay has been developed due to the difficulties in obtaining sufficient amounts of human relaxin as described in the Introduction to this thesis and in the Final Discussion. However, a homologous porcine relaxin radioimmunoassay which appears to recognise human relaxin has been described by O'Byrne and Steinetz (1976). This assay using the same antibody was set up in the author's laboratory for the studies described in this thesis. The ratio of biological to immunological activity is 1.3-4.4 to 1 (O'Byrne et al 1978).

4.2 REAGENTS

(1) Assay Buffer 0.5 phosphate buffered saline, pH 7.4, with 5% bovine serum albumen.

(2) Standard Porcine Relaxin Pure porcine relaxin (B31) as purified in Chapter 2 of this thesis.

(3) Control Plasma Ovariectomised female plasma from the same species as the unknown samples.

(4) Antibody Rabbit anti-porcine relaxin serum (R6 as kindly supplied by Dr. E.M. O'Byrne, New York). The cross-reactivity and specificity of this antibody for relaxin-like substances in the blood of various mammalian species including the pig and the human have been published (O'Byrne and Steinetz 1976 & O'Byrne et al 1978).

(5) Antibody Diluent Buffer 0.05M of ethylenedinitrilo-tetraacetic acid, pH 7.4 with 2% normal rabbit serum.

(6) Relaxin Derivative for Radio-iodination N-α-formyl-tyrosyl-relaxin was kindly supplied by Dr. Christian Schwabe, U.S.A. As discussed in the Introduction, porcine relaxin does not have any iodinatable amino acid residues and therefore an iodinatable relaxin derivative must be synthesised. N-α-formyl-tyrosyl-relaxin has been found to be a reliable and reproducible tracer for relaxin radioimmunoassay (Schwabe 1983).

(7) Second Antibody Goat antibody to rabbit gamma-globulin (Calbiochem) was used. 125 units were dissolved in 25 ml of assay buffer.

4.3 PROCEDURES

(1) Radio-iodination of the Relaxin Derivative 1 milli Curie of I^{125} was added to 5 µg of N-α-formyl-tyrosyl-relaxin per 5 µL of distilled water followed by freshly prepared chloramine T solution (50 µg in 10 µL of 0.05M phosphate buffer, pH 7.5). After shaking for 10 seconds, freshly prepared sodium metabisulfite (100 µg in 10 µL phosphate buffer) was added followed immediately by potassium iodide solution (1 mg in 100 µL phosphate buffer). Separation of the iodinated relaxin from the free NaI^{125} was accomplished by gel filtration on a 0.5 x 20 cm column of Sephadex G-25 (medium) presaturated with 2% bovine gamma globulin in 0.05M phosphate buffer, pH 8.6 and eluted with phosphate buffer. Fractions of 0.5 ml were collected and the fractions with the highest binding as measured in the RIA were used.

(2) Radioimmunoassay Procedure All glassware used during the procedure was siliconised to reduce any binding of relaxin to the glass and all assays were performed in triplicate.

(a) Standard porcine relaxin in quantities of 0, 1, 4, 16, 64, 256 and 1024 ng/ml diluted to 200 µL with assay buffer was added to the standard curve tubes.

(b) 200 µL of assay buffer alone was added to 100 µL of the samples to be assayed and a similar amount of buffer was added to non-specific binding control tubes which contained 200 µL of antibody diluent buffer.

(c) An equal volume (200 µL) of control plasma was added to the standard curve tubes and the non-specific binding control tubes.

(d) Antibody (R6) in quantities of 200 µL at a dilution of 1 in 5,000 in diluent buffer was added to the standard curve tubes and unknown samples bring the final volume in each tube to 600 µL.

(e) The solutions were mixed gently and incubated for 24 h at $4^{o}C$.

(f) 100 µL of radio-iodinated relaxin (containing 25,000 counts per minute) was added to each tube in the assay, gently mixed and incubated for a further 24 h at 4°C.

(g) On the third day, 200 µL of second antibody was added to each tube, mixed and again incubated at 4°C for 24 h.

(h) Thereafter, the tubes were centrifuged at 2,500 r.p.m. at 4°C. The supernatant was siphoned off and the remaining pellet was counted in a Beckman gamma-counter. The relaxin concentration in the unknown samples were determined by interpolation from a least square regression line of logit (B/B_o) versus log concentration of a standard relaxin according to the linear equation of Rodbard et al (1969).

$$\text{Logit } (B/B_o) = b. \log (X) + a$$

where B is counts bound, B_o is counts bound for zero dose, X is the hormone concentration, a and b are constants. N (non-specific counts) determined by the omission of the antibody to the non-specific binding control tubes were subtracted from the B and B_o before logit transformation.

4.4 DETAILS AND VALIDATION OF ASSAY

A standard curve obtained during this radioimmunoassay is shown in Figure 14.

The dose-response curve was linear over the range of 1 to 64 ng/ml. The mean slope of the dose-response curve was b = 2.25 (n = 10). The least detectable dose as defined by two standard deviations of percentage binding at the zero point was approximately 12.5 pg of relaxin. The between-assay and the within-assay coefficients of variation of the radioimmunoassay were 38.0% (n = 10) and 9.5% (n = 10) respectively. Porcine and human insulin, prolactin, FSH, LH and TSH showed no cross-reactivity with this assay.

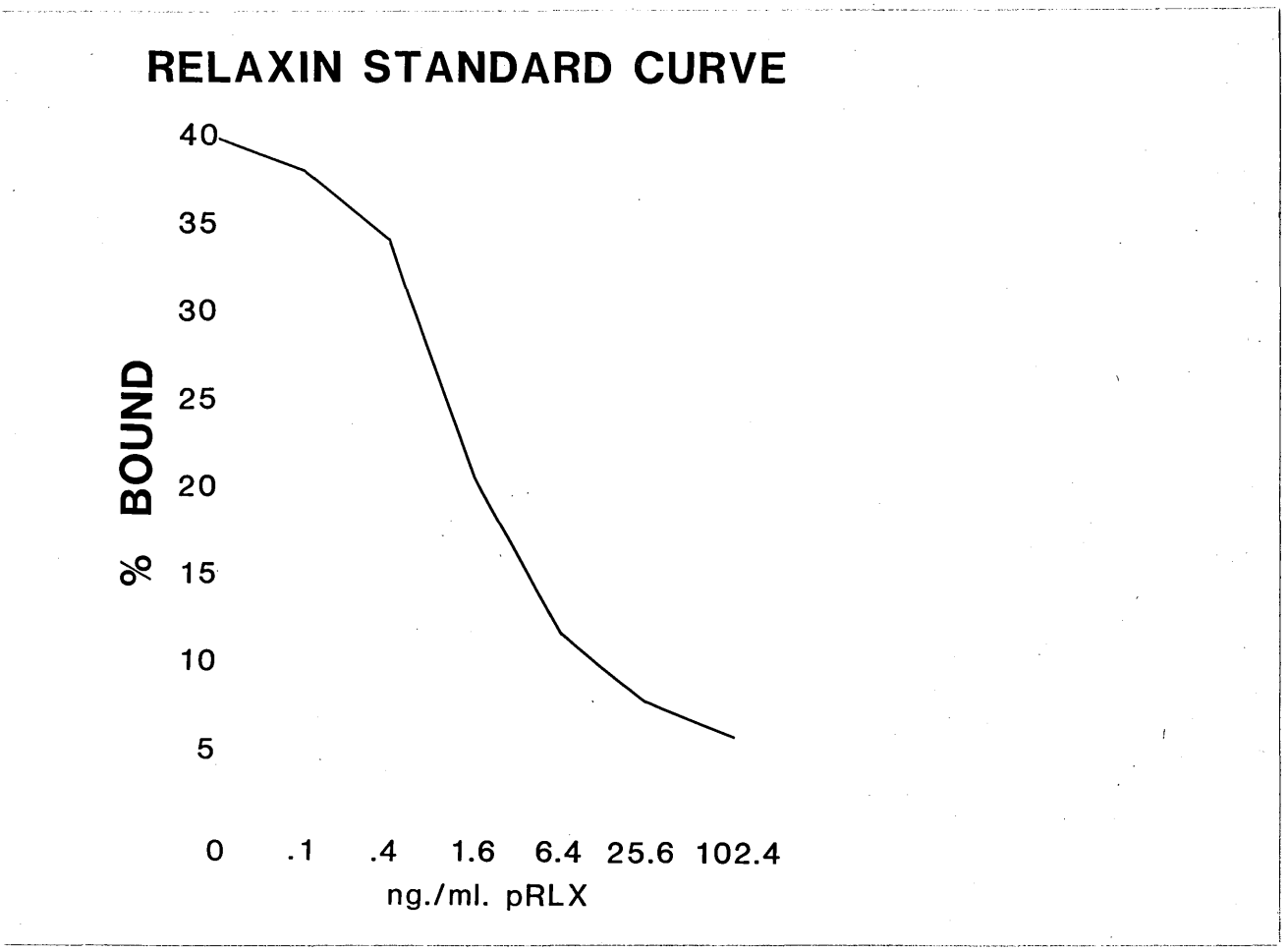


FIGURE 14 A standard curve obtained during the radioimmunoassay of porcine relaxin used in these studies.

CHAPTER 5

SERUM RELAXIN LEVELS IN NORMAL HUMAN PREGNANCY

5.1 SUMMARY

In a cross sectional study of 330 normal singleton human pregnancies the relaxin-like immunoactivity in peripheral serum was measured in a porcine relaxin radioimmunoassay. Serum relaxin concentrations were highest in early pregnancy and gradually declined until term. At term, patients in labour had a significantly higher mean relaxin concentration compared to patients who were not in labour. No significant rise in relaxin levels could be detected in patients sampled during the week prior to the spontaneous onset of labour. After term there was a continuing significant decline in relaxin concentrations in patients who were not in labour. By the third postnatal day relaxin concentrations had fallen almost to the level of the non-pregnant control group. This pattern of relaxin secretion is compatible with the postulated roles of relaxin during human pregnancy with regards the maintenance of myometrial quiescence, the facilitation of uterine stromal remodelling during uterine growth and the promotion of cervical ripening at the onset of parturition.

5.2 INTRODUCTION

In mammals it is postulated that the polypeptide hormone relaxin promotes connective tissue remodelling during reproduction, inhibits myometrial contractility until late pregnancy and facilitates the progress of cervical ripening at the initiation of parturition (MacLennan 1983). Relaxin is secreted from the human corpus luteum of pregnancy but may also be produced by the human decidua and the basal plate of the placenta (Larkin, Pardo & Renegar 1983). Human relaxin has recently been characterised but is not yet available for the development of a human relaxin radioimmunoassay. However, a homologous radioimmunoassay using porcine relaxin antisera has been established (O'Byrne & Steinetz 1976). There have only been a few reports (O'Byrne et al 1978; Quagliarello et al 1979; Szlachter et al 1982; Scarselli et al 1983) of this assay's application in the human. In most animals there is a rise in serum relaxin levels prior to or at the beginning of parturition but as yet this has not been described in the human. The purpose of this study is to establish relaxin levels throughout normal human pregnancy as measured in a porcine relaxin radioimmunoassay and to compare these levels to those obtained in other human and animal studies using similar assays.

5.3 PATIENTS AND METHODS

Permission for this study was obtained from the Research and Ethics Committee of The Queen Victoria Hospital, Adelaide and all samples were obtained with informed patient consent. Three hundred and thirty serum samples were collected throughout pregnancy from 6 to 42 weeks gestation and on the third postnatal day. Eleven further control samples were collected from non-pregnant healthy premenopausal patients. The samples collected during pregnancy were grouped in weekly brackets and only samples from patients with a normal singleton pregnancy and a gestational age confirmed by early ultrasound were included in the study. The study was a cross sectional study and the number of samples per individual patient ranged from 1 to 4. Forty-four of the patients between 38 and 41 weeks laboured spontaneously and the timing between sampling and the onset of labour was calculated in these patients.

All samples consisted of 10 ml of clotted blood which was centrifuged and the serum frozen at -80°C for subsequent radioimmunoassay. The antibody used was R6 rabbit antiporcine relaxin which has been used to measure immunoreactive relaxin in women (O'Byrne & Steinetz 1976), the tracer used was N-α-formyl-tyrosyl-relaxin (Schwabe 1983) and the second antibody was goat antibody to rabbit gammaglobulin (Calbiochem). The sensitivity of the assay was 12.5 pg. The between-assay and the within-assay coefficients of variation of the radioimmunoassay were 38 and 9.5 percent respectively. The data are expressed as nanogram per millilitre equivalents of porcine relaxin. The mean values for each gestation have been calculated $\pm$ standard deviation of the mean. Groups were compared by the Student (two tailed) t-test.

5.4 RESULTS

Serum levels of relaxin during human pregnancy as measured in the porcine relaxin radioimmunoassay are shown in Figure 15. Relaxin levels (mean ± S.D.) appeared to rise early in pregnancy and the levels were higher in the first half of pregnancy (6-20 weeks) (0.69 ± 0.31 ng/ml) than in the second half of pregnancy (21-40 weeks) (0.48 ± 0.18 ng/ml) ($p<0.001$). Patients who werenot in labour at 41 and 42 weeks gestation had significantly lower mean serum relaxin levels (0.28 ± 0.10 ng/ml) than the levels recorded during the second half of pregnancy ($p<0.001$). Overall relaxin levels declined throughout pregnancy (6-40 weeks) and a line of regression with a significant negative correlation was obtained ($r= -0.42$) ($p<0.001$).

Patients in labour had a significantly higher mean ± S.D. relaxin level (0.59 ± 0.33 ng/ml) than either the mean relaxin level of patients in the second half of pregnancy ($p<0.05$) or patients at 40 weeks gestation (0.39 ± 0.13 ng/ml) ($p<0.01$). The highest relaxin levels during labour were recorded in patients in early labour. Of the 44 patients sampled near term to determine relaxin levels close to spontaneous parturition, 24 spontaneously laboured within one week of the sampling and their individual levels are shown in Figure16, plotted by days prior to parturition. The mean ± S.D. relaxin level of this group was 0.36 ± 0.14 ng/ml and was not significantly different from the mean of the remaining 20 patients who did not labour within one week of sampling (0.35 ± 0.15 ng/ml). No significant trend was noted in the relaxin levels in the days prior to labour but only two samples were obtained within 24 h of the spontaneous onset of labour.

Samples obtained on the third postnatal day (mean ± S.D. 0.19 ± 0.15 ng/ml) were significantly lower than during the second half of pregnancy ($p<0.001$) as were the control samples from the non-gravid

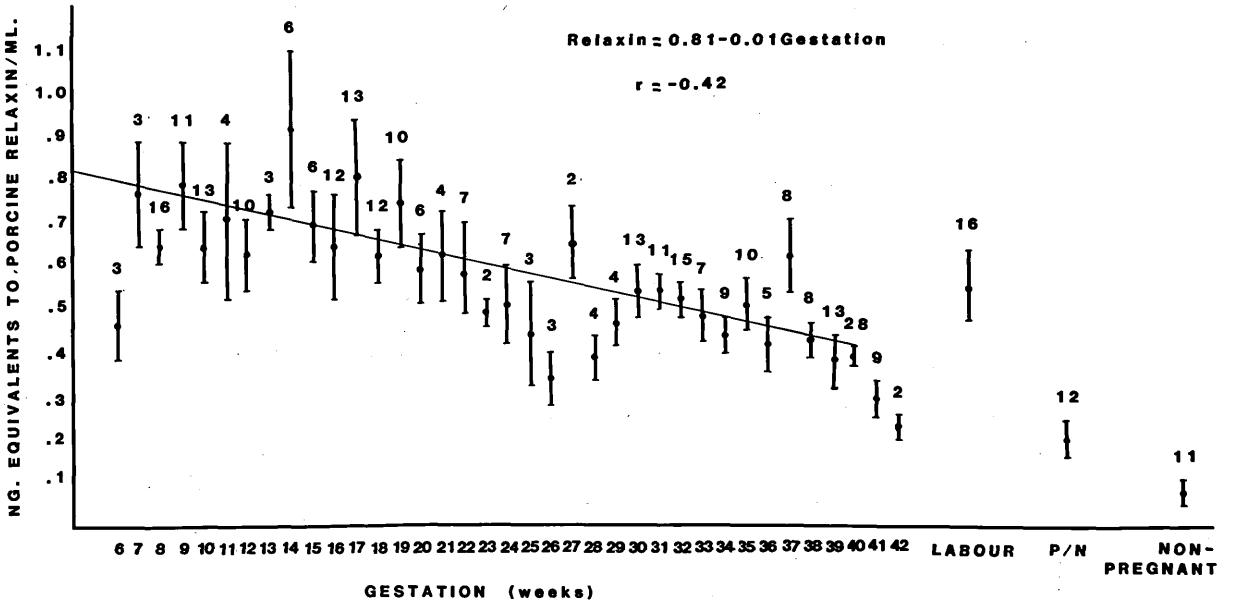


Figure 15 Mean immunoreactive relaxin levels (± S.E.) in the serum of women throughout the course of pregnancy until the third postnatal day. The line of regression (r) has been plotted and mathematically expressed.

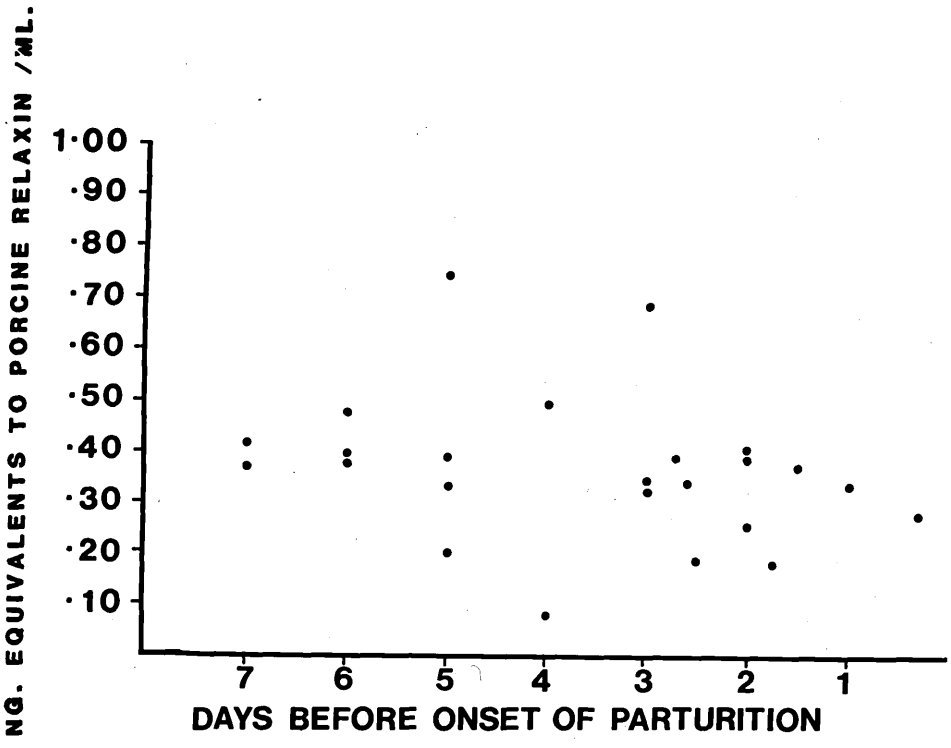


Figure 16 Individual serum relaxin concentrations in patients who laboured within 7 days of sampling plotted by days prior to the onset of spontaneous labour.

patients (mean $\pm$ S.D. 0.08 $\pm$ 0.1 ng/ml) ($p<0.001$).

No obstetric pathology or abnormal obstetric outcome could be correlated with the pregnancies in which high or low relaxin levels of relaxin were obtained.

5.5 DISCUSSION

The pattern of peripheral relaxin levels in early and mid pregnancy in this study closely matches the two main published studies of O'Byrne et al (1978) and Quagliarello et al (1979) both of which used a similar porcine relaxin radioimmunoassay. The actual levels (in porcine equivalents) of relaxin in this study are very similar to those published by O'Byrne et al (1978). Quagliarello et al (1979) reported levels approximately two-fold higher than those in this study. Both these previous studies suggested that relaxin levels remained stable in the third trimester after a drop in mid pregnancy, whereas the data in this study suggests there is a continuing slow decline in peripheral serum relaxin levels after a peak is reached early in the second trimester. Serum relaxin levels dropped significantly further after term in this study and this finding is in keeping with the report by Szlachter et al (1982) who found a reduction in serum relaxin in pregnancies beyond 43 weeks gestation. The rapid drop in relaxin levels in the postnatal period is consistent with the findings of other studies and was not affected by lactation.

A rise in peripheral relaxin levels has been described in almost every species studied prior to parturition but no previous human study has shown a rise in serum relaxin before or during labour. This is the first human study to show a significant rise in peripheral relaxin levels at parturition. However, this was a cross sectional study and the findings should be confirmed in a serial study. There are practical difficulties in anticipating spontaneous human parturition and obtaining frequent serial samples around this time. Ideally serial samples should be collected hourly rather than daily as the rise in serum relaxin may be very brief. The cross sectional study described in this paper failed to show any significant rise during the week prior to labour. Serial

daily samples from 3 patients sampled one to four days prior to labour by Quagliarello et al (1979) also showed no rise in relaxin levels. In this study the highest levels were obtained in very early spontaneous labour and the results of adequate serial sampling in the human at this time have yet to be published.

The significantly higher relaxin levels found in the patients in early labour compared with their term non-labouring counterparts is in keeping with the pattern in other animals where there is a surge in relaxin immediately before or during early labour. This surge in relaxin correlates with an increase in cervical softening and dilatation in most animals and it is postulated that one of relaxin's main roles is to facilitate parturition by promoting connective tissue remodelling in the cervical tissues. In the human, exogenous porcine relaxin applied vaginally at term can induce such cervical ripening and in some instances initiate parturition (MacLennan et al 1980).

Further postulated roles for relaxin are that it may facilitate uterine stromal remodelling allowing growth and distension of the uterus and that along with progesterone, it may promote uterine quiescence during pregnancy by inhibiting myometrial contractility (Chapter 10). The pattern of its secretion as described in this paper would be compatible with such roles, the levels of relaxin being highest in early pregnancy and gradually declining until parturition. In the rat relaxin inhibits both spontaneous and prostaglandin induced myometrial contractility but does not inhibit oxytocin induced myometrial contractility (Porter, Downing & Bradshaw 1979). The synthesis of human relaxin is awaited to see if this is also true in the human. Should a similar mechanism of action be shown, then a rise in relaxin levels at parturition facilitating cervical ripening would not inhibit myometrial contractility in a uterus responsive to oxytocin due to the induction of oxytocin receptors in the myometrium at term.

The significance of the findings in this study depends on the validity of the heterologous application of this homologous porcine radioimmunoassay and on whether peripheral serum levels truly reflect local activity at the site of action. There is only approximately a 50 percent homology in the amino acid sequences between porcine and human relaxins (Hudson et al 1983). The synthesis of human relaxin and the development of a human relaxin radioimmunoassay is awaited. However, until then, in trying to define the role of relaxin in human reproduction, it is useful to have an understanding of the possible pattern of relaxin secretion during normal pregnancy and to look for changes in these patterns in pathological pregnancies, e.g. premature labour.

CHAPTER 6

THE RELATIONSHIP OF THE HORMONE RELAXIN TO PELVIC PAIN AND JOINT LAXITY IN HUMAN PREGNANCY

6.1 SUMMARY

Peripheral serum relaxin levels were measured in a porcine relaxin radioimmunoassay in 17 patients with severe pelvic pain and pelvic joint instability during late pregnancy. These results were compared to a control group of 330 samples obtained throughout pregnancy from normal singleton pregnancies. The majority of the serum relaxin concentrations of the study group were above but within 1 S.D. of the mean for the corresponding gestational age of the control group. The mean relaxin concentration (0.63 ng/ml) of the patients with pelvic symptoms was significantly higher than the mean relaxin concentration of the normal patients during the second half of pregnancy (0.48 ng/ml) ($p<0.005$). Three patients had relaxin concentrations more than 2 S.D. from the mean of the normal patients for the corresponding gestational age and these patients were amongst the most clinically disabled. The three highest levels of relaxin in the study group were higher than any in the 163 patients in the control group sampled during the second half of pregnancy. This study suggests that in some patients higher than normal relaxin levels may be associated with and be responsible for excessive pelvic joint laxity and pelvic pain in late pregnancy.

6.2 INTRODUCTION

A major degree of separation of the pubic rami due to increased compliance of the connective tissues in the pubic symphysis happens only in species such as rodents where the ratio of the diameters of the maternal pelvis and the fetal head is too small to allow normal delivery without such a separation. In the human the ratio of these diameters is sufficiently large that separation of the pubic symphysis is not normally necessary. It has been estimated, using radiological techniques, that there is only a mean 7 mm increase in vertical stretching and a mean 3 mm increase in lateral stretching of the human pubic symphysis during pregnancy (Farbrot 1952). However, a minority of women in pregnancy experience severe pelvic pain in both the pubic symphysis and the sacroiliac joints. A proportion of these patients have clinically apparent joint instability and laxity and they may be unable to walk during the end of pregnancy. There have been no specific studies on pelvic joint laxity in pregnancy and radiological studies are not now acceptable. However, increased peripheral joint laxity has been reported in the third trimester of pregnancy with an increase in laxity associated with multiparity (Calguneri, Bird & Wright 1982).

In rodents such as the guinea-pig and the mouse there is a rise in serum relaxin prior to the onset of parturition which is associated with separation of the pubic symphysis (Hisaw 1926). This separation can also be induced by the injection of exogenous relaxin in the oestrogen-primed animal. However, no correlation has yet been published between relaxin levels in the human and pubic symphysial pain and laxity. The aim of this study was to measure peripheral serum relaxin levels in patients with major degrees of pelvic joint pain and laxity during late pregnancy and compare these results to the levels

found in normal pregnancy using the same porcine relaxin radioimmuno-assay (Chapter 5).

6.3 PATIENTS AND METHODS

Permission for this study was obtained from the Research and Ethics Committee of The Queen Victoria Hospital, Adelaide. Patients were included in the study only when their complaint of pelvic joint pain was sufficiently incapacitating to necessitate prolonged bed rest in hospital or at home. Most of these patients had major problems in walking and all cases were assessed by a physiotherapist who performed lateral flexion tests with the patient standing on alternate legs. During these tests pubic symphysial pain was reproduced or exacerbated and joint instability was apparent. An accurate clinical scoring system to assess the degree of pelvic pain and joint instability could not be validated. However, only patients with severe incapacitating pelvic joint pain associated with clinically apparent joint instability have been included in the study. A single serum sample was obtained from 16 patients, and three antenatal samples were obtained from a further patient who appeared to be more severely incapacitated than the other patients, in that she was hospitalised for the last 14 weeks of her pregnancy and was unable to walk during that time. Postnatal samples were obtained from three of the patients. Only the mean antenatal relaxin levels of the patient sampled more than once has been included in calculating the mean serum relaxin level of the total group. Students (two tailed) t-test was used to compare the mean relaxin level of the pelvic pain group to that in normal pregnancy as assayed in the same radioimmunoassay (Chapter 5). The assay used was a homologous porcine radioimmunoassay as described previously (Chapter 4) using R6 rabbit antiporcine relaxin serum for the antibody (O'Byrne & Steinetz 1976), N-α-formyl-tyrosyl-relaxin as the tracer (Schwabe 1983) and goat antibody to antirabbit gammaglobulin (Calbiochem) as the second antibody. The

within assay coefficient of variation was 9.5 percent and the sensitivity of the assay was 12.5 pg. All samples from the patients with pelvic pain were measured in the same radioimmunoassay together with control samples from patients in the normal pregnancy group.

6.4 RESULTS

The serum relaxin concentrations in patients with pelvic pain are shown in Figure 17 and are compared to the levels previously described in normal pregnancy (Chapter 5). The mean ($\pm$ S.D.) relaxin concentration of the patients with pelvic joint pain was 0.63 $\pm$ 0.25 ng/ml and was significantly higher than the mean concentrations of relaxin in normal patients during the second half of pregnancy (0.48 $\pm$ 0.18 ng/ml) ($p<0.005$). The relaxin concentration in only 1 pelvic pain patient was below the mean for the second half of pregnancy in the normal group. Three study patients had levels more than 2 S.D. above the corresponding mean of the control group. These three patients had relaxin levels higher than any of the 163 samples taken from normal singleton pregnant patients during the second half of pregnancy. The patient who was thought clinically to have the greatest degree of pelvic joint pain and instability had the highest relaxin levels (1.15 to 1.39 ng/ml). In the three patients sampled postnatally relaxin concentrations had returned to normal non-pregnant levels.

Amongst the 163 samples obtained between 21 and 40 weeks gestation from normal pregnancies only 5 samples had relaxin concentrations (range 66 to 86 ng/ml) which were greater than 2 S.D. from the mean for that gestation. None of these patients complained of major degrees of pelvic pain and none required hospitalisation for pelvic problems.

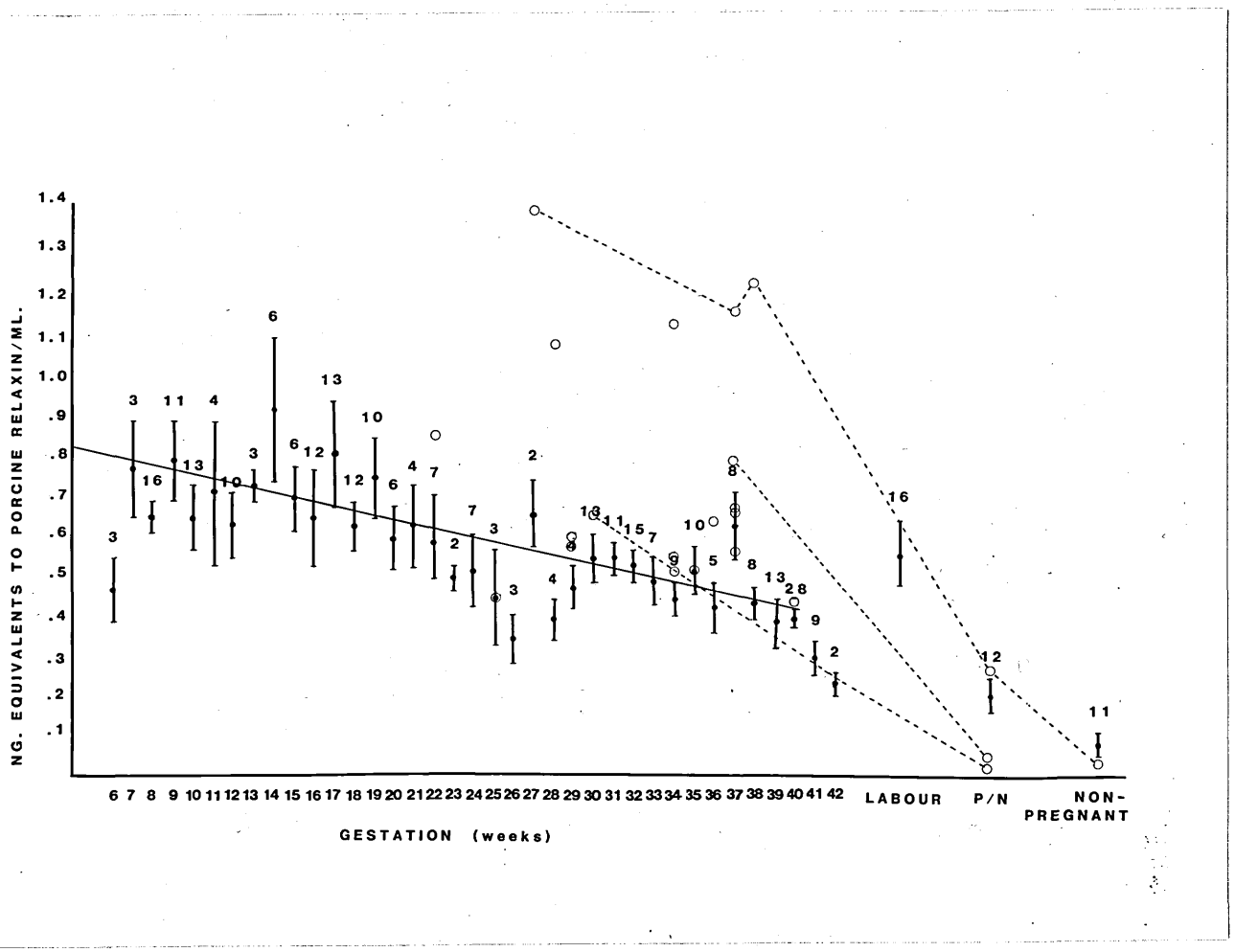


FIGURE 17 Serum relaxin concentrations in 17 patients with pelvic joint pain and laxity (o) plotted in comparison to the mean (± S.E.) levels at each week of gestation found in normal patients (Chapter 5). Samples from the same patient are connected by dotted lines (red).

6.5 DISCUSSION

Although the mean serum relaxin concentration in the patients with pelvic pain and joint laxity was significantly higher than the control group, only 3 patients had very high serum relaxin concentrations. However, only 1 patient in the study group had a relaxin concentration below the mean for the control group during the second half of pregnancy. Moderately high levels of relaxin, e.g. 66 to 86 ng/ml in the control group were not associated with major evidence of pelvic symptoms, hospitalisation or incapacity on retrospective analysis of their obstetric history. Minor degrees of pelvic pain and joint laxity were not assessed in this study but clearly not all patients with higher than normal relaxin levels in pregnancy complain of pelvic joint instability and pain. Equally not all pregnant patients with pelvic pain have clinically apparent joint laxity and there are many other causes of such pain, e.g. nerve compression, urinary tract infection, etc. A difficulty experienced in this study was the quantification of pain and pelvic joint laxity and a quantitative scoring system of these symptoms and signs could not be validated. Nevertheless, the patient with the highest relaxin levels required the longest hospitalisation and the other 2 patients with the very high serum relaxin levels required admission to hospital and were unable to walk at the end of their pregnancy. All gradually recovered postnatally. In the 3 patients sampled postnatally the relaxin levels had fallen to levels below the sensitivity of the assay.

Peripheral serum relaxin levels may not be as relevant as relaxin receptor levels and it may be that some patients are more susceptible to circulating or local concentrations of relaxin if a high level of receptors have been induced in the connective tissue of the pubic symphysis and sacroiliac joints. This might explain why the

patients in the control group who had moderately high relaxin levels did not have symptoms. No human studies have been performed on relaxin receptors in these tissues but relaxin receptors have been described in fibroblasts taken from the mouse pubic symphysis (Bryant-Greenwood 1982). The induction of relaxin receptors is thought to be under the influence of oestrogen. The increase in the width of pubic symphysis in the mouse during pregnancy involves the erosion of cartilage and bone and the transformation of the hyaline cartilage caps to fibrous connective tissue. These morphological changes in the pubic symphysis can be experimentally reproduced by the injection of relaxin if the mouse has been previously primed with oestrogen (O'Byrne et al 1982).

This study shows that some patients with pelvic pain and laxity of their pelvic joints have much higher than normal peripheral serum relaxin levels as measured in a porcine radioimmunoassay during the second half of pregnancy and suggests that these high relaxin levels may be responsible for their excessive pelvic joint instability and pain during the latter months of pregnancy. The validity of relaxin-like immunoactivity in human serum as measured in a porcine relaxin radioimmunoassay requires confirmation when a specific human relaxin radioimmunoassay is available. Thereafter, studies are required to determine why relaxin levels are high in patients with pelvic pain and excessive joint laxity and whether relaxin levels can be safely lowered, e.g. with antiprostaglandin agents, with clinical effect.

CHAPTER 7

RELAXIN LEVELS IN MULTIPLE PREGNANCY, PREMATURE LABOUR, PRE-ECLAMPSIA, NORMAL NEONATES AND NEONATES WITH CONGENITAL DISLOCATION OF THE HIP.

7.1 SUMMARY

Peripheral serum relaxin levels, as measured in a porcine relaxin radioimmunoassay, in normal singleton pregnancies were compared to relaxin concentrations in the serum from patients with multiple pregnancy, premature labour and pre-eclampsia and to serum relaxin concentrations from the umbilical cord of normal neonates and neonates with congenital dislocation of the hip. The mean relaxin concentration (0.70 ng/ml) of 11 patients with a multiple pregnancy sampled in late pregnancy was significantly higher ($p<0.001$) than the mean for normal singleton pregnancies at the same gestation (0.48 ng/ml). Eight patients in premature labour had a significantly lower mean relaxin concentration (0.29 ng/ml) than the control group who were not in premature labour ($p<0.005$). The mean relaxin concentration of 14 patients with moderate to severe pre-eclampsia was not statistically significant from the control group. Umbilical cord serum samples from both normal neonates and those with congenital dislocation of the hip were all measured as being below the sensitivity of the assay. This suggests that there is little transfer of maternal relaxin to the fetus and that an association between high fetal relaxin levels and congenital dislocation of the hip could not be substantiated.

7.2 INTRODUCTION

There have been very few reports of relaxin concentrations in abnormal pregnancy. Szlachter et al (1982) reported that relaxin levels in third trimester multiple pregnancies and in pre-eclampsia were not different from normal pregnancy but were lower in patients in premature labour. Whilst it has been postulated that congenital dislocation of the hip may be caused by high relaxin levels, relaxin levels have never been studied in this condition. In this study peripheral serum relaxin concentrations were measured in four perinatal pathologies using the same homologous porcine relaxin radioimmunoassay and compared to the concentrations of relaxin found in normal singleton pregnancy as described in Chapter 5.

Relaxin levels were measured in the following circumstances:

(a) multiple pregnancy

(b) premature labour

(c) pre-eclampsia, and

(d) umbilical cord samples from normal neonates and neonates with congenital dislocation of the hip.

There has only been one report in the literature of the measurement of relaxin in fetal blood (Weiss et al 1978). In that study, using a similar heterologous application of a porcine relaxin radioimmunoassay, relaxin was only just detectable in 1 of 10 cord blood serums tested. The sensitivity of that assay was reported as 50-100 pg. As the sensitivity of the radioimmunoassay reported in this study was lower (12.5 pg) 40 cord blood sera were studied in normal pregnancies with a normal neonatal outcome and 18 cord blood sera were examined in babies who were found to have unilateral or bilateral congenital dislocation of the hip in the neonatal period.

Although the aetiology of this latter condition is un-

known there is suggestive evidence that hormonal factors during pregnancy may cause fetal hip joint laxity and interfere with the normal development and formation of the fetal hip joint (Wilkinson 1963).

7.3 METHODS AND RESULTS

Multiple pregnancy

One serum sample was obtained from each of ten twin pregnancies and one triplet pregnancy between 30 and 38 weeks gestation. The results are shown in Table 2 and Figure 18. The mean (± S.D.) concentration of relaxin in these multiple pregnancies was 0.70 ± 0.21 ng/ml. This was significantly higher than the mean concentration (0.48 ± 0.18 ng/ml) of relaxin in normal singleton pregnancies during the second half of pregnancy ($p<0.001$).

Premature labour

A single serum sample was obtained from 8 patients in premature labour. Premature labour was defined as regular contractions, as recorded by an electronic abdominal pressure transducer, occuring every ten minutes or less associated with effacement and dilatation of the cervix before the completion of the 37th week of pregnancy. Other pregnancy complications frequently occur with this group of patients and they are recorded in Table 4 along with the time of delivery in relation to the time the serum samples were obtained.

The serum relaxin concentrations of patients in premature labour are shown in Table 3 and they were all below the regression line for patients with normal ongoing pregnancies (Figure 19). The mean relaxin concentration (± S.D.) of the premature labouring patients was 0.29 ± 0.14 ng/ml. This was statistically significantly lower than the mean relaxin concentration for the control group during the second half of pregnancy ($p<0.005$).

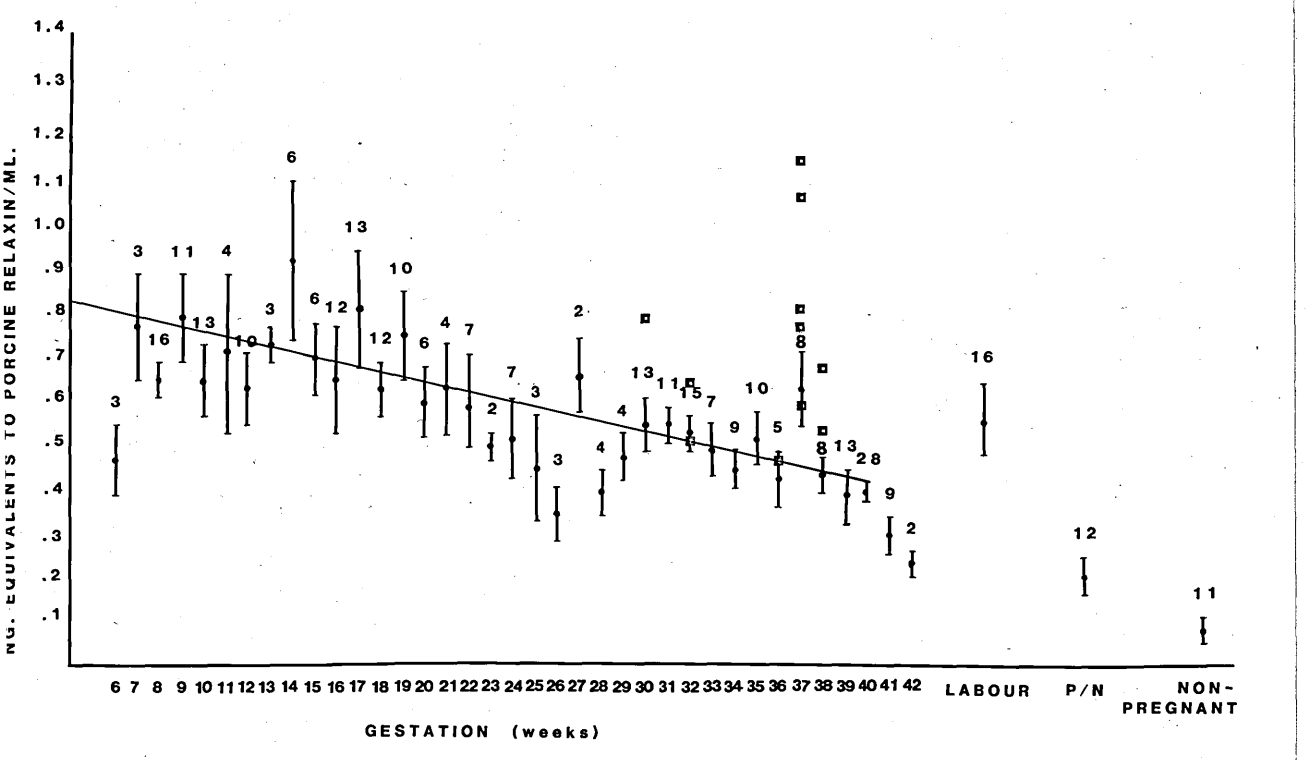


FIGURE 18 Serum relaxin concentrations in patients with a multiple pregnancy (red squares) plotted in comparison to the mean (± S.E.) levels at each week of gestation found in normal singleton pregnancy (Chapter 5).

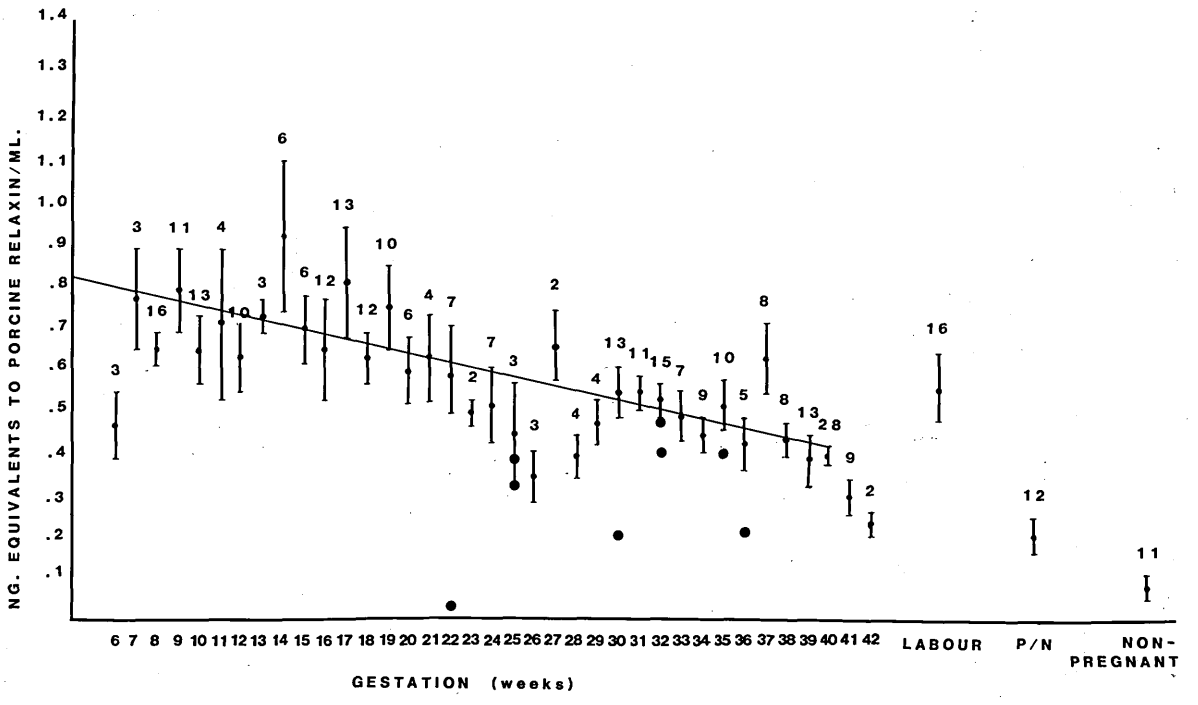


FIGURE 19 Serum relaxin concentrations in patients in premature labour (blue) plotted in comparison to the mean (± S.E.) levels at each week of gestation found in normal pregnancy (Chapter 5).

PREGNANCY	GESTATION	RELAXIN CONCENTRATION ng/ml x 100
Twin	30	75
Twin	32	62
Twin	32	50
Twin	34	112
Twin	36	45
Twin	37	57
Twin	37	79
Twin	37	75
Twin	37	103
Twin	38	52
Triplet	32	50

TABLE 2 Individual serum relaxin concentrations in 11 multiple pregnancies sampled during the third trimester

PREGNANCY COMPLICATIONS In relation to date of sampling	GESTATION At sampling	RELAXIN CONCENTRATION ng/ml x 100
Intrauterine death approximately 2 days prior to sampling	22	2
Antepartum haemorrhage one week prior to sampling. Delivered live baby one day later.	25	37
Antepartum haemorrhage on day of sampling. Delivered stillborn child five days later.	25	31
Delivered live baby 6 hours after sampling.	30	19
Delivered by caesarean section 2 hours after sampling.	32	39
Premature labour inhibited for 3 further weeks.	32	46
Delivered a growth retarded baby 8 hours after sampling.	35	38
Delivered 2 days later	36	20

TABLE 3: Individual serum relaxin concentrations in 8 patients defined as being in premature labour at the time of sampling.

Pre-eclampsia

A single serum sample was obtained from 14 patients with moderate or severe pre-eclampsia between 29 and 38 weeks gestation. In this study pre-eclampsia was defined as pregnancy induced hypertension greater than 100 mm Hg diastolic blood pressure, associated with proteinuria and/or oedema. The results are shown in Table 4. The mean (± S.D.) concentration of relaxin in the pre-eclamptic patients was 0.56 ± 0.16 and was not statistically different from the mean of the normal control patients in the second trimester.

Umbilical cord levels in normal neonates and neonates with congenital dislocation of the hip

Relaxin concentrations in both groups ranged from zero to 0.12 ng/ml and thus all the relaxin concentrations were below the sensitivity of the assay. The mean ± S.D. for the normal babies was 0.048 ± 0.041 ng/ml and the mean for the babies with congenital dislocation of the hip was 0.043 ± 0.044 ng/ml. Statistical analysis was not performed as all the levels were below the sensitivity of the assay.

GESTATION	RELAXIN CONCENTRATION ng/ml x 100
29	56
31	59
32	67
32	68
32	60
33	38
34	36
34	70
35	69
36	86
37	30
37	43
38	63
38	39

TABLE 4 Individual serum relaxin concentrations in 14 singleton pregnancies with moderate to severe pre-eclampsia

7.4 DISCUSSION

Multiple pregnancy

In this study of third trimester multiple pregnancies 10 of the 11 patients had serum relaxin concentrations above the mean of the control singleton pregnancies at the same gestation. Although in the only other report of relaxin levels in multiple pregnancy Szlachter et al (1982) did not find a statistically significant rise in the relaxin concentration of twin pregnancies in the third trimester they did report high levels in 2 triplet pregnancies and very high levels in one mid trimester pregnancy before the patient aborted 9 fetuses.

The results of the study reported in this thesis and the later data of Szlachter et al suggest that serum relaxin concentrations in the human are related to the amount of corpora luteal tissue present and possibly the amount of placental tissue present as this is another site of relaxin production. In the sow, Martin et al (1979) has shown that serum relaxin concentrations are related to the number of corpora lutea present and the results in this human study are in keeping with the findings in the pig. However, serial samples from early in multiple pregnancy would help to confirm or refute the data in this study.

Premature labour

As will be discussed in later chapters relaxin in conjunction with progesterone inhibits myometrial contractility during some or all of pregnancy in the rat and the pig. In these animals relaxin appears to play a major role in maintaining uterine quiescence during pregnancy. Access to human relaxin is awaited to test the hypothesis that relaxin in the human also

inhibits myometrial contractility until late in pregnancy. It is, therefore, of interest to note that many of the serum relaxin concentrations in patients in premature labour were low and all were below the mean for the control group at similar gestations. This is in agreement with the findings of Szlachter et al (1982). Only 8 patients in premature labour have been sampled so far in this study as this is the beginning of a further 3 year study and baseline levels have had to be established for normal non-labouring patients. The study of premature labour also presents major problems for the clinical researcher as firstly the definition of clearly established progressive premature labour can be difficult and secondly many patients with premature labour have other associated pathologies as can be seen in the patients sampled in this study. Therefore the collection of large numbers of patients for this study will take time. There will be merit in future studies in measuring the serum progesterone and oestrogen as well as serum relaxin to look for correlations in the ratios of these hormones and premature labour. In particular, a specific human relaxin radioimmunoassay will be helpful to validate any possible association with low relaxin levels and premature labour. However, these initial pilot studies using the homologous porcine radioimmunoassay suggest that there may be such an association.

Pre-eclampsia

No difference was found in the mean relaxin concentration of patients with pre-eclampsia and normal patients. This is in keeping with the findings of the only other similar report by Szlachter et al (1982) and suggests that there is no apparent link between abnormal relaxin secretion and pre-eclampsia. It

also suggests that the finding of lower relaxin levels in other abnormal obstetric conditions is likely to be specific to that condition rather than a non-specific effect of pre-eclampsia when that condition is coincidentally present.

Umbilical cord levels

In the only other report of relaxin concentrations in the fetus at birth, relaxin could only just be detected in 1 out of 10 cord samples and 1 out of 9 amniotic fluid samples (Weiss et al 1978). The results of this study of 40 normal neonates is in agreement with that study and suggests that very little maternal relaxin crosses into the fetal circulation. It also suggests that the fetus does not secrete relaxin unless it is a relaxin of different structure that is not recognised by the porcine relaxin radioimmunoassay.

Higher than normal levels of relaxin were not found in the cord serum of neonates diagnosed as having congenital dislocation of the hip. However, it should be noted that all levels recorded in both normal and abnormal neonates were below the sensitivity of the assay and no definite conclusion can be reached that relaxin does not influence the normal formation of the fetal hip joint. Only if relaxin levels in the affected neonates had been significantly above both the levels in the normal neonates and the sensitivity of the assay could a possible association have been postulated. However, further studies using a more sensitive and specific human radioimmunoassay are still warranted.

CHAPTER 8

EFFECTS OF RELAXIN ON THE INTRAUTERINE DISTRIBUTION AND ANTIMESOMETRIAL POSITIONING AND ORIENTATION OF RAT BLASTOCYSTS PRIOR TO IMPLANTATION

8.1 SUMMARY

An intravenous infusion of porcine relaxin was administered to rats from the afternoon of day 4 of pregnancy to the morning of day 6, a time by which implantation has normally occurred. Over the same time period control rats received intravenous saline. At sacrifice, implantation sites in the control rats were evenly distributed throughout the length of each uterine horn on their antimesometrial borders. In the relaxin treated rats the implantation sites were irregularly distributed and confined to the cranial half of each uterine horn. Histological sections of uteri fixed by vascular perfusion with osmium tetroxide revealed that the blastocyst was no longer invariably positioned anti-mesometrially within the lumen and that embryonic disc orientation was often abnormal. A reduced decidual cell reaction was observed around several of the implanting blastocysts.

8.2 INTRODUCTION

In the rat, implanting blastocysts are distributed more or less evenly along each uterine horn, with implantation always being initiated at the antimesometrial border of the uterine lumen and the embryonic disc within each blastocyst always orientated towards the mesometrium (Finn 1977). However, the mechanisms responsible for the distribution, positioning and orientation of blastocysts along each uterine horn are poorly understood. Migration of the embryonic disc within the trophoblast has been discounted by Gardner (1975), while other workers have shown that differential uterine growth around each implantation site could be responsible for the more even spacing of such sites (see e.g. Finn 1968). Rogers, Murphy and Gannon (1982) demonstrated that such differential uterine movement may also be achieved by tissue hydration, resulting from the increase in endometrial capillary permeability which occurs at each implantation site. McLaren & Michie (1959) postulated that myometrial activity was responsible for the even distribution of the blastocysts, before implantation, from the utero-tubal junction along each horn. Evidence to support this theory comes from the work of Pusey et al (1980) who used an intravenous infusion of porcine relaxin (a potent inhibitor of myometrial activity in the rat) to disrupt normal distribution before implantation, resulting in the majority of the blastocysts being recovered from the cranial third of each uterine horn. The present work was undertaken as a preliminary study to determine whether the continuation of such a relaxin infusion would result in the majority of implantation sites being similarly confined to the cranial third of each uterine horn, and to examine the effects of relaxin on the normal antimesometrial positioning and orientation of the blastocysts at implantation.

8.3 MATERIALS AND METHODS

Female Porton rats (262-327 g) showing a pro-oestrous vaginal smear were housed overnight with a male; spermatozoa in the smear the following morning confirmed this day as day 1 of pregnancy. Each rat was fitted with an indwelling jugular vein cannula (Silastic tubing, i.d. 0.635 mm o.d. 1.194 mm; Dow Corning, Midland, Michigan, U.S.A.) under methohexitone anaestheisa (3.3 ml brietal sodium/Kg, 2% solution in water, i.p.; Eli Lilly (Australia), Sydney) on the morning of day 3 of pregnancy. The cannula was run s.c. to the top of the head and exteriorized via a dental cement headpiece held in place by 3 watchmakers screws fixed into the skull. Rats were housed individually in environmentally controlled cages. The exteriorized cannula (polyethylene tube, i.d. 0.86 mm, o.d. 1.52 mm; Dural Plastics, New South Wales 2158, Australia) was run up to the roof of the cage inside a protective coiled wire spring. At the point where the cannula passed through the roof of the cage a swivel was incorporated in the line to permit it to rotate without kinking. An infusion pump (IM-1, Narashige, Tokyo, Japan) housed outside the cage, and calibrated to deliver 0.2 ml/h was connected to the end of the cannula.

Relaxin was obtained from the ovaries of pregnant sows and was purified by the method of Walsh & Niall (1980), giving highly purified B31 porcine relaxin, which had a biopotency of 1500 GPU/mg relative to the reference preparation NIH-R-PI (potency 442 GPU/mg). (Steinetz et al 1960).

Two rats were treated as controls, receiving a saline (9 g NaCl/L) infusion from 17:00 h on day 4 of pregnancy to 10:00 h on day 6. Environmentally controlled cages were set for onset of light at 07:30 h and onset of darkness at 19:30 h. Six rats received relaxin infusions

(6.66 µg/h) in saline water over the same time period).

At 15 min. before autopsy, the rats were given an intravenous injection of 1% Evans blue in saline, via the indwelling jugular cannula, to permit identification of implantation sites (Rogers et al 1982). As soon as the relaxin infusion was completed the rats were anaesthetised with intravenous pentobarbarbitone sodium (40 mg Nembutal/Kg; Abbot, Sydney), the descending thoracic aorta was rapidly cannulated and the renal arteries were approached through separate lateral abdominal incisions and clamped shut with haemostats at points close to each kidney. Osmium tetroxide (50 ml of 1% aqueous solution) in 0.1 M-phosphate buffer pH 7.4 containing 9 g NaCl/L was infused via the thoracic cannula, the first 40 ml with a driving pressure of 150 mmHg and the last 10 ml at 50 mmHG. The primary osmium fixation took about 10 min. This was followed by vascular washout of the osmium fixative with 50 ml 0.1 M-phosphate buffer pH 7.4 containing 9g NaCl/L at 100 mm Hg driving pressure, and finally with 50 ml 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 containing 9 g NaCl/L at 100 mmHg. The whole uterus was then carefully excised with a minimum of physical twisting or bending, and further fixed by immersion in 2.5% glutaraldehyde for 1 h. The whole uterus was rinsed and then stored in 0.1 M-phosphate buffer pH 7.4, before being processed for routine light microscope histology.

8.4 RESULTS

Implantation sites in the control rats were evenly distributed along the full length of each uterine horn (Fig. 20 (1)). However, in the rats that had received a relaxin infusion the implantation sites (as indicated by the bands of blue uterine tissue) were always confined to the cranial half of each uterine horn (Fig. 20 (2)). The number of sites per horn ranged from 2 to 6 (mean 4.5) and their distribution was variable, with blue bands appearing from almost continuous to well separated. In one instance, two implanting blastocysts were found within 120 μm of each other, with the primary decidual reactions being continuous.

A histological section of a normal rat implantation site from the morning of day 6 of pregnancy, showing the antimesometrial positioning of the blastocyst, and the surrounding primary decidual reaction, is illustrated in Figure 21 (3). By comparison examples of implantation sites taken at the same stage of pregnancy, but after an intravenous infusion of relaxin, are shown in Figure 21 (4-6). The details of blastocyst position and orientation from relaxin treated rats are summarized in Table 5. Two of the treated rats could not be included in the experimental data because the uterine lumen opened during either fixation or histological processing, dislodging the blastocysts, and thus making it impossible to assess their luminal position or orientation accurately.

Despite the apparently normal positioning of 11 of the blastocysts near the antimesometrial border of the lumen, 5 of these 11 showed abnormal embryonic disc orientation (Fig. 21 (4)). In most of these 5 cases the embryonic disc was laterally rather than mesometrially directed, although in at least one blastocyst the disc was orientated fully towards the antimesometrial border. Each of the three blastocysts

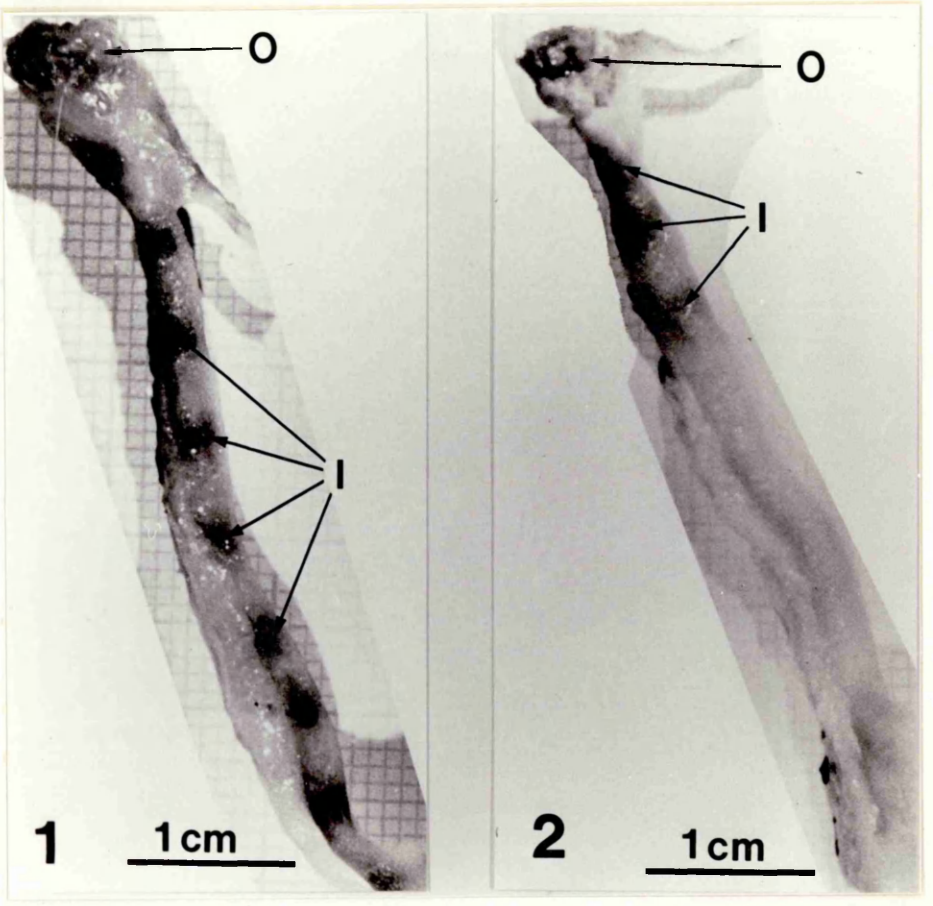


Figure 20-1 Uterine horn from a control rat killed on the morning of Day 6 of pregnancy 15 min after receiving an i.v. injection of Evans blue. Note the relatively even spacing of the implantation sites (I) (represented by the dark bands where Evans blue bound to serum albumin has accumulated in the tissues due to the locally increased endometrial vascular permeability around each implantation site) along the full length of the uterine horn. O, ovary.

Figure 20-2 Uterine horn from a rat killed on the morning of Day 6 of pregnancy after receiving a continuous infusion of porcine relaxin from the afternoon of Day 4 untilthe time of death. Note how the implantation sites (I) are irregularly spaced and confined to the cranial half of the uterine horn. O, ovary.

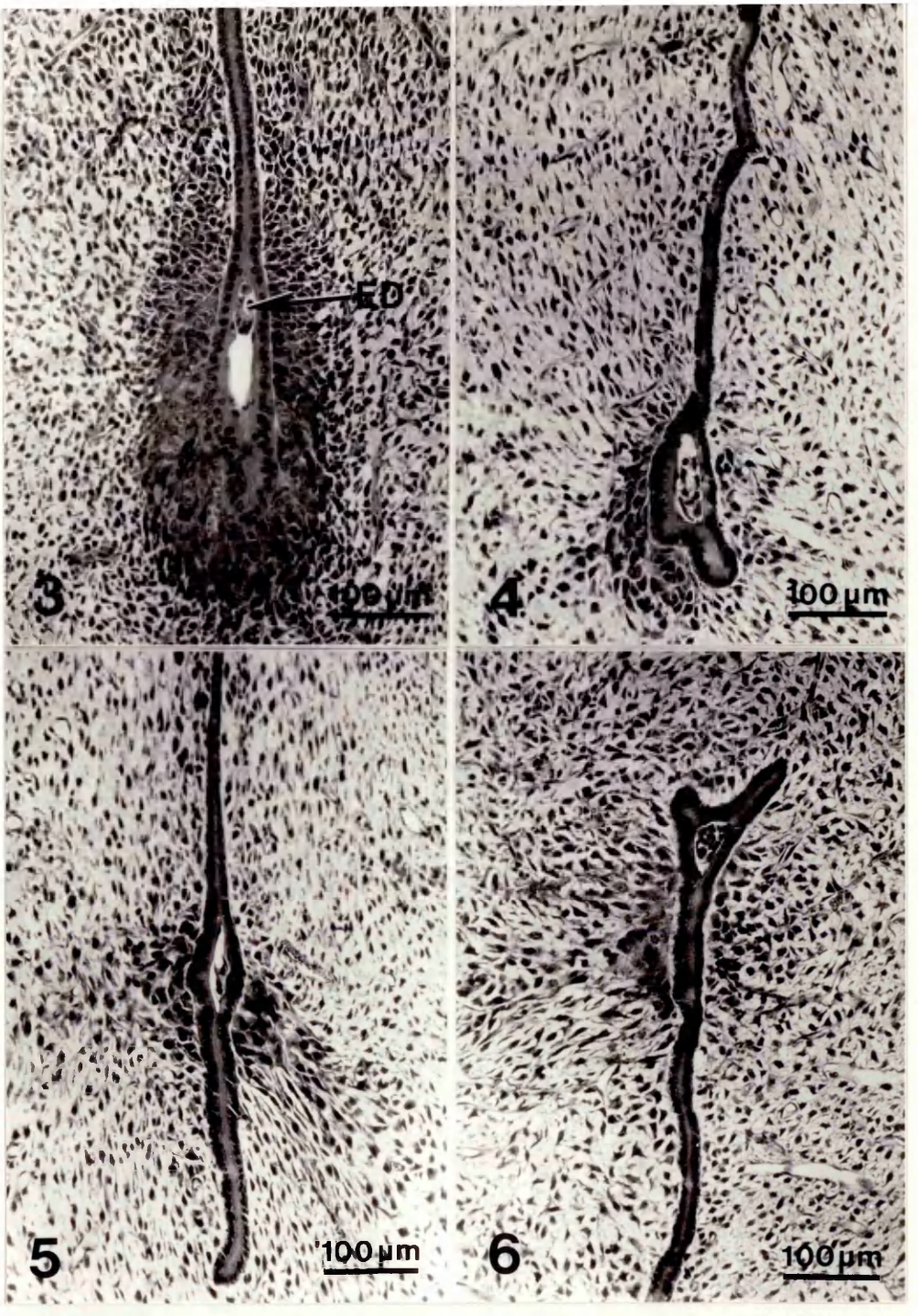


Figure 21-3 Histological section of a control implantation site taken on the morning of Day 6 of pregnancy. The blastocyst is positioned anti-mesometrially within the uterine lumen with the embryonic disc (ED) orientated towards the mesometrium, while the surrounding stromal cells exhibit an early decidual reaction. H & E. 6µm.

Figures-4-6 Histological sections of implantation sites from relaxin treated rats on Day 6 of pregnancy. The antimesometrial margin is at the bottom in all photographs. In each example the embryonic disc is not orientated towards the mesometrium, and in Figs 5&6 the blastocyst is not positioned at the antimesometrial border of the lumen. Note the reduced decidual reactions compared with that in Fig. 3, and the differences in epithelial thickness on the mesometrial compared with the antimesometrial side of the blastocyst in Fig. 5. H & E, 6µm.

NO. OF IMPLANTING BLASTOCYSTS	DISTANCE OF IMPLANTATION SITE FROM A-M BORDER (μm)	DISTANCE FROM A-M BORDER AS % OF TOTAL DISTANCE BETWEEN A-M AND MESOMETRIAL LUMINAL BORDERS	NO. OF BLASTOCYSTS IN EACH GROUP SHOWING NON-MESOMETRIAL ORIENTATION OF THE EMBRYONIC DISC
11	0-100	0-9%	5
3	101-200	10-13%	0
1	260	21%	1
1	700	44%	1
1	930	89%	1
1	1080	62%	1

TABLE 5 Data for blastocyst positioning and orientation relative to the antimesometrial (A-M) border of the uterine lumen on day 6 of pregnancy in 4 rats treated with relaxin.

that had implanted 101-200 μm from the antimesometrial border showed normal embryonic disc orientation. The remaining 4 blastocysts had abnormal embryonic disc orientation.

The extent of the decidual reaction appeared markedly reduced in many of the implantation sites from relaxin treated rats compared with controls (e.g. compare Fig. 21 (3) with Fig. 21 (4-6)). However, the degree of decidualization shows large variability even in normal circumstances, and so due to the limited number of specimens available, quantitative studies of the size or extent of the decidual reaction in relaxin treated rats were not undertaken in this study.

In several of the implantation sites located up to 260 μm from the antimesometrial border, the luminal epithelium mesometrial to the blastocyst appeared different from that on the antimesometrial side (Fig. 21 (5)), the mesometrially located epithelium was thinner than that on the antimesometrial side of the blastocyst, with individual cells appearing cuboidal rather than columnar. No measurements of epithelial thickness were taken in this study due to the limited numbers of specimens available.

8.5 DISCUSSION

This work demonstrates that blastocysts are able to initiate implantation successfully under the influence of exogenous relaxin, and confirms that the distribution of the implantation sites on day 6 of pregnancy is similar to that of the preimplantation blastocysts under the influence of relaxin on day 5 of pregnancy, i.e. restricted to the cranial portion of each uterine horn. It also demonstrates that under the influence of relaxin, rat blastocysts do not invariably implant at the antimesometrial border of the uterine lumen, and also that the embryonic disc is not always orientated towards the mesometrium. To our knowledge this is the first report of non-antimesometrial implantation and/or unusual embryonic disc orientation in the rat, and the technique is therefore useful to investigate further these complex and poorly understood phenomena.

That 11 of the 18 blastocysts sectioned from relaxin treated rats were positioned at or near the antimesometrial border of the lumen suggests that the normal antimesometrial positioning mechanism may still have been at least partly effective. This may be due either to the relaxin dose being only marginally effective, or a more indirect effect. It is possible, for example, that the unusual crowding of all the blastocysts at the ovarian end of the uterine horn due to the effects of the relaxin on the myometrium (Pusey et al 1980), may disrupt the subsequent antimesometrial positioning mechanism. Of the 11 blastocysts positioned antimesometrially, 5 had unusual embryonic disc orientation, suggesting that the mechanism responsible for orientation may be, in part at least, different from that responsible for antimesometrial positioning. This would also explain the observation that 3 of the blastocysts that did not achieve normal antimesometrial positioning did, however, show normal embryonic disc orientation. A

minimum of three separate mechanisms may therefore be involved in normal blastocyst movement before implantation; the first in spacing the blastocysts along the uterine horn, which involves the myometrium, the second in moving the blastocysts to the antimesometrial border, and the third in orientating the blastocysts so that the embryonic disc points towards the mesometrium.

The 4 blastocysts that implanted a substantial distance from the antimesometrial border all showed unusual embryonic disc orientation. Despite this, these sites demonstrate that it is possible for implantation to be initiated well away from the antimesometrial border, and that it is the blastocyst, and not the antimesometrial portion of the endometrium, which is responsible for initating decidualization.

The differences in epithelial thickness mesometrial to the blastocyst compared with antimesometrially, as seen in some implantation sites (Fig. 21 (5)), may be an indication of one of the mechanisms responsible for blastocyst movement. It is possible that these morphological differences seen histologically represent epithelial cell migration in the living tissue. Thus the epithelium may be involved in moving the blastocyst towards its "correct" position, even though it has already implanted, by migrating towards the antimesometrial border.

CHAPTER 9

THE EFFECT OF PORCINE RELAXIN VAGINALLY APPLIED AT HUMAN EMBRYO TRANSFER IN AN IN VITRO FERTILISATION PROGRAMME

9.1 SUMMARY

It has been suggested that the polypeptide hormone relaxin is an early pregnancy factor which facilitates implantation and pregnancy maintenance. To test this hypothesis a double blind randomised placebo controlled trial was conducted where 2 mg purified porcine relaxin or distilled water was given in a vaginal gel on the day of embryo transfer and again three days later in a human in vitro fertilisation (IVF) programme. There were 96 patients in the randomised trial and 73 patients who were treated concurrently in the same IVF programme acted as a further control group. Of the 51 patients who received relaxin, 10 pregnancies were confirmed and 8 continued successfully. In the 45 patients treated with placebo 10 pregnancies were also confirmed and 6 continued successfully. Amongst the 73 patients concurrently treated outside the trial 14 achieved a pregnancy and 10 continued to term. Thus, porcine relaxin given in these circumstances in a human IVF programme did not appear to improve or interfere with the pregnancy rate. Possible factors that affected the implantation rates in this trial are discussed.

9.2 INTRODUCTION

The fertilised ovum spends 3 to 4 days in the fallopian tube prior to implantation in the uterus about 7 days after ovulation. During this time messages are transmitted from the human blastocyst to the host to prepare the endometrium for implantation (Psychoyos 1973), to promote the secretion of uteroglobin (Beier 1982) and to facilitate an increase in the activity of the corpus luteum (Lenton et al 1982). The messages involved in the communication of the presence of a pregnancy are unknown but human chorionic gonadotrophin, human placental lactogen and schwangerschaftsprotein 1 may play such a role (Klopper 1983).

The polypeptide hormone relaxin can be detected soon after ovulation (Thomas, Loumaye & Ferin 1980). Plasma levels of relaxin increase if conception occurs and are at their highest during early pregnancy (Quagliarello et al 1979). In 1967, Hisaw et al (1967) showed that in the monkey the development of the full histological changes of the endometrium in early pregnancy depended on the presence of three hormones in combination, i.e. oestrogen, progesterone and relaxin. Relaxin appeared to augment the progestational changes in the endometrium and induce hyperplasia of the endometrial blood vessel endothelium, a charactertistic of normal decidualisation. It has been postulated that relaxin may be another luteal factor facilitating early pregnancy maintenance and, in particular, implantation through its potential to induce connective tissue remodelling in target tissues (MacLennan 1981).

During the process of in vitro fertilistion there is no opportunity for messengers of early embryonic origin to inform the mother or the corpus luteum of the successful fertilisation until the embryo is transferred directly into the uterus, usually some 40-72 hours after

oyum pick up. Thus, should these messengers induce secretion of relaxin or other luteal factors for early pregnancy maintenance, there may be a delay in their production and such a mistiming might be responsible for the relatively low pregnancy rates of around 16-29 percent (Edwards & Steptoe 1983) currently being achieved following human embryo transfer.

The continuous infusion of pure porcine relaxin in the rat over the time of implantation interferes with the normal distribution of the multiple blastocysts down the uterine horn and causes them to implant only in the cranial half (Pusey et al 1980; Rogers et al 1983). Such an effect of exogenously administered relaxin after the transfer of one or two human blastocysts to the upper uterine cavity might be potentially advantageous.

Since 1974 it has been possible to produce highly purified relaxin from porcine sources and this purified form of relaxin has been successfully used without side effects or antibody formation in clinical trials of cervical ripening near term (MacLennan et al 1980; MacLennan et al 1981; Evans et al 1983). In these trials the relaxin was given in a vaginal tylose gel and increasing peripheral blood levels of relaxin were detectable within one hour after application. Although there are many possible factors that influence the successful outcome of pregnancy following the transfer of embryos arising from oocytes fertilised in vitro, the failure of approximately 80 percent of apparently normal embryos to implant after embryo transfer warranted a clinical trial of the administration of exogenous relaxin at the time of embryo transfer to determine if this potential early pregnancy maintenance factor given vaginally could improve the pregnancy rate.

9.3 PATIENTS AND METHODS

Relaxin was obtained from the ovaries of pregnant sows and was purified by the method of Sherwood and O'Byrne (1974). The relaxin used in this study had a biopotency of 1500 guinea pig units (GPU) /mg relative to the reference preparation NIH-R-PI (potency 442 GPU/mg). The purified relaxin used was equivalent to the CM-a^1 peak described by Sherwood and O'Byrne (1974) and is the same as that used for the analysis of the primary structure and sequence studies of porcine relaxin (James et al 1977).

Permission for the clinical trials was obtained from the research and ethics committee of the Queen Elizabeth Hospital, Adelaide. A double blind randomised placebo controlled trial was proposed and the number of patients to be included in the trial was determined statistically by calculating the number required to be 80 percent sure of seeing a significant ($p<0.05$) increase in the pregnancy rate. A doubling of the pregnancy rate in the treatment group was sought over the pregnancy rate current at the beginning of the trial and a sample number of 96 was determined to be sufficiently large to have such a chance of showing this trend.

After obtaining informed patient consent the patients were randomly allocated, using a list of random numbers, on a double blind basis to receive either 2 mg purified porcine relaxin or a placebo (distilled water) mixed with 300 mg of water-soluble cellulose (Tylose) granules (Hoechst) to make a viscous gel. The appropriate gel was placed in the posterior vaginal fornix by means of a soft latex catheter and a syringe immediately after embryo transfer and again three days later. Pregnancies were confirmed initially with serum Beta HCG levels and later by ultrasound. Statistical analysis of the results was performed using the χ^2 test.

Ninety-six patients consented to be in the double blind randomised trial which took place over a 15 month period. Throughout this time a further 73 patients were treated in the same in vitro fertilisation programme and were only excluded from the trial for administrative reasons, e.g. the temporary absence of one of the research team or the non availability of relaxin. These 73 patients, who did not receive any form of vaginal gel, are included in the results as a further control group.

9.4 RESULTS

During the 15 months period of this trial there were a total of 280 cycles tracked, 211 laparoscopies and 169 embryo transfers. The pregnancy outcomes of the treatment and control groups are shown in Table 6. The pregnancy rates in each group are not statistically significantly different from each other ($p>0.05$).

The pregnancy incidence and sequence of successful implantation in all groups during the trial was non uniform with pregnancies often occuring in groups between which there were lengthy sequences where no pregnancy was achieved. The clusters of pregnancies did not correlate with the relaxin treatment but statistical analysis suggests that such groupings of the pregnancies are unlikely to have happened by chance.

The number of embryos transferred also influenced the pregnancy rate. One to four embryos were transferred and the number of embryos transferred and the corresponding pregnancy rates for the relaxin and placebo treated groups are shown in Table 7.

There were 8 ongoing multiple pregnancies in the total of 24 successful pregnancies achieved during the 15 month period studied. There were 4 sets of twins in the placebo group and 1 set of triplets in the relaxin treated group. Thus, in the relaxin and placebo groups, there were 7 and 2 ongoing singleton pregnancies respectively.

No side effects were noted in any of the 96 patients treated with either relaxin or placebo. One patient in the placebo group miscarried at 16 weeks in association with a uterine rupture through a previously scarred uterine horn.

OUTCOME	RELAXIN GROUP (n = 51)	PLACEBO GROUP (n = 45)	CONCURRENT CONTROL GROUP (outside study) (n = 73)
Confirmed pregnancies	10	10	14
Pregnancies that miscarried	2	3 + 1 ectopic	4
Number of continuing pregnancies (>20/52)	8	6	10

TABLE 6 Pregnancy outcome after embryo transfer in relaxin and placebo treated patients.

NUMBER OF EMBRYOS TRANSFERRED	RELAXIN GROUP			PLACEBO GROUP		
	Not Pregnant	Continuing pregnancy	Miscarried	Not pregnant	Continuing pregnancy	Miscarried
1	11	1	0	11	0	2
2	19	5	0	14	2	1
3	10	0	2	6	2	0
4	1	2	0	4	2	1

TABLE 7 Pregnancy rate in treatment and placebo groups according to number of embryos transferred.

9.5 DISCUSSION

Porcine relaxin, given vaginally in 2 mg doses at human embryo transfer and three days later, does not appear to improve or interfere with the pregnancy rate following in vitro fertilisation and embryo transfer. Limitations on the availability of pure porcine relaxin and the numbers of patients in the IVF programme did not allow the number of patients in the trial to be large enough to detect possible more subtle influences on singleton pregnancy. It has already been shown that multiple embryo transfer is likely to be more successful than single embryo transfer (Kerin et al 1983) and it is possible that the endometrium and ovary recognise the multiple stimulus of several embryos more readily than one. Four of the six ongoing pregnancies in the placebo group were multiple gestations.

Human pregnancy is possible even in patients without ovaries (Lutjen et al 1983) and thus ovarian relaxin cannot be essential for pregnancy maintenance. However, there are other sources of relaxin in human pregnancy, e.g. the decidua (Bigazzi et al 1983) which could be a significant source of this hormone. Since this trial commenced the amino acid sequence of human relaxin has been deduced (Hudson et al 1983). There is only approximately a 50 percent homology between the amino acid sequences of human and porcine relaxin. Thus, although porcine relaxin has been shown to be moderately effective in inducing cervical ripening in the human (MacLennan et al 1980; Evans et al 1983) it is possible that human relaxin receptor sites in the endometrium (as in human myometrium - see Chapter 10) may not fully recognise porcine relaxin. The dose of relaxin given in the trial and the timing of its administration were by necessity empirically chosen and are, therefore, other potential factors influencing the apparent lack of effect of relaxin in these circumstances. Finally, in all in vitro fertilisation

programmes at present there are many clinical and laboratory variables which may influence the pregnancy outcome, such as the culture conditions prior to embryo transfer, the quality and numbers of the embryos transferred and the degree of synchrony of the endometrial changes at the time of implantation. The non-uniform distribution of pregnancies during the in vitro fertilisation programme suggests that unknown factors other than relaxin influenced the success of the programme from time to time. When these factors are better understood and controlled there may be justification to repeat a similar clinical trial using pure human relaxin, when this becomes available in the near future using genetic engineering techniques.

CHAPTER 10

THE EFFECT OF PORCINE RELAXIN AND PROGESTERONE ON RAT, PIG AND HUMAN MYOMETRIAL ACTIVITY IN VITRO

10.1 SUMMARY

The *in vitro* effect of pure porcine relaxin on non-pregnant and pregnant myometrium before and after progesterone treatment was studied in the rat, pig and human. Myometrial strips from 45 rats, 30 pigs and 64 humans were studied. Porcine relaxin inhibited myometrial activity in the non-pregnant and pregnant rat until mid pregnancy when the sensitvity to relaxin gradually decreased. By day 19 of rat pregnancy the myometrium was completely refractory to the inhibitory effect of porcine relaxin. Pretreatment of rat myometrial strips *in vitro* with progesterone increased their sensitivity to relaxin but did not overcome the refractory period in late pregnancy. In the pig myometrial contractility was completely inhibited by porcine relaxin both in the non-pregnant state and throughout pregnancy (days 25-109). Progesterone was again synergistic with relaxin in the pig in inhibiting myometrial contractility. However, porcine relaxin had little or no effect on human myometrial contractility either in the non-pregnant state or during the gestations of pregnancy tested (28-42 weeks). Pretreatment of human myometrium with progesterone could not induce a response to porcine relaxin. It is suggested that the lack of response of human myometrium to porcine relaxin may be due to the non-recognition of porcine relaxin by human myometrial relaxin receptors because of the large difference in structure between porcine and human relaxin.

10.2 DISCUSSION

One of the principle actions of the polypeptide hormone relaxin is the inhibition of myometrial activity (Porter 1979b; MacLennan 1983). This action was first described in oestrogen-primed guinea-pigs by Krantz et al in 1950 using very impure preparations of relaxin. These porcine preparations were less than 10 percent pure and did not inhibit human myometrial activity in vitro (Miller, Kisley & Murray 1957). Despite the negative in vitro findings, clinical trials using such impure preparations of relaxin were conducted during the 1950s and gave inconclusive and conflicting results. Recent biochemical advances have allowed the complete purification of porcine relaxin (Sherwood & O'Byrne 1974; Walsh & Niall 1980) and it has been possible to study the effect of porcine relaxin on myometrial activity without the influence of contaminants.

The effect of porcine relaxin on rat myometrium has been well described and is the basis of one of the bioassays for this hormone (Wiqvist 1959; St. Louis 1981). The non-pregnant rat has to be oestrogen-primed for relaxin to exert its inhibitory effect on myometrial activity and in pregnancy its effect in vitro can only be found during the first half of pregnancy. Progesterone and porcine relaxin are synergistic in decreasing the amplitude of non-pregnant rat myometrial contractions in vitro (Sarosi et al 1983) and it has been postulated that these hormones in combination play an important part in maintaining uterine quiescence during pregnancy. However, a synergistic effect of these hormones during pregnancy in the rat has not yet been described.

There is no literature on the effect of pure porcine relaxin on human myometrium in either the pregnant or non-pregnant state, nor have there been such reports of the effect of pure porcine relaxin on pig

myometrial activity where, of course, the relaxin would be species specific. Although extracts of human corpora lutea of pregnancy have been tested on human myometrial activity in vitro with an inhibitory effect (Szlachter et al 1980; Beck et al 1982) human relaxin has not yet been purified in sufficient quantities to examine its specific myometrial effect *in vivo* or *in vitro*.

This study was designed to comprehensively study and clarify the effect of pure porcine relaxin on gravid and non-gravid myometrial activity in the rat, pig and human and examine its potential synergism with progesterone.

10.3 METHODS

Rat

Thirty-two Hooded Wistar non-pregnant oestrogen-primed female rats (weight 200-250 G) were studied. Each rat received 0.2 mg of oestradiol valerate subcutaneously 20 hours prior to decapitation. Thirteen pregnant rats were also studied. One was sacrificed on day 10, 4 on day 14, 1 on day 17 and 7 on day 19 of pregnancy. One of the day 14 rats had a unilateral pregnancy and both the pregnant and non-pregnant uterine horns were studied.

Pig

Seven mature cycling female non-pregnant pigs and 23 pregnant pigs at varying gestations (25-109 days) were studied.

Human

Permission for collection of human myometrium was granted by The Queen Victoria Hospital, Adelaide, Research and Ethics Committee. Forty-seven strips of myometrium were obtained at caesarean section (gestations 28-42 weeks) where after delivery of the baby a thin 2 cm long strip of myometrium was excised from the upper edge of the incision in the lower uterine segment. Similar pieces of myometrium were exicsed from 17 non-pregnant hysterectomy specimens obtained from both premenopausal and post-menopausal patients.

Relaxin

Relaxin was obtained from the ovaries of pregnant sows and was purified by the method of Sherwood and O'Byrne (1974) with the modifications of Walsh and Niall (1980). The relaxin used in

this study had a biopotency of 1500 guinea-pig units (GPU) /mg relative to the reference preparation NIH-R-PI (potency 442 GPU/ mg). The purified relaxin used was equivalent to the CM-a' peak described by Sherwood and O'Byrne (1974) and is the same as that used for the analysis of the primary structure and sequence studies of porcine relaxin (James et al 1977).

Experiment

After excision the uterine biopsies were placed in Earle's medium. Myometrial strips 2 mm diameter x 2 cm long were prepared from the biopsies and immediately suspended by cotton thread in a 15 ml double jacketed organ bath, containing Earle's medium. The lower end of the myometrial strip was attached to a clamp at the bottom of the bath and the other end was connected by the thread to a Transmed Isometric Force Transducer Model No. T-1030. The contractile activity was isometrically recorded with a Gilson polygraph, calibrated at an initial tension of 5G for pig and human myometrium and 1G for rat myometrium.

The composition of Earle's medium used in these experiments in millimoles per litre was $CaCl_2$, 1.4; glucose, 5.6; $MgSO_4$, 0.4; KCl, 5; NaCl, 117; NaH_2PO_4 ^2H_2O, 0.9. The organ bath was kept at a constant temperature of 37°C and a pH of 7.4 was maintained by a constant flow of 95 percent O_2 and 5 percent CO_2. The organ bath was siliconised prior to use to reduce adhesion of relaxin to the glassware. Porcine relaxin concentrations in the medium were confirmed by a porcine relaxin radioimmunoassay utilising rabbit anti-porcine relaxin serum R6 and ^{125}I-labelled poly-tyrosyl-relaxin (O'Byrne & Steinetz 1976).

Spontaneous contractions were recorded without interference until both the strength and the rate of contractions were steady.

Increasing doses of relaxin were then added to the organ bath to assess the effect on muscle contractility. To determine whether the original activity of the muscle could be restored the relaxin was washed out with fresh Earle's medium. If this was not achieved the experiment was discarded.

KCl-induced contractions were produced according to the bioassay of St. Louis (1981). A 20 percent KCl substitution of the normal NaCl content in Earle's medium, induces a tetanic contraction of myometrium giving a stable plateau on the polygraph recording. The quantitative effect of a relaxant can be measured as a percentage of the reduction in the height of the plateau. In all cases the effect of porcine relaxin was studied on spontaneous myometrial activity and using the KCl-bioassay of St. Louis (1981).

Progesterone

Two myometrial strips taken from the same uterine biopsy from rat, pig or human, were placed in separate organ baths. One strip was pretreated for 45 minutes with progesterone in propylene glycol (15 µl) to give a final concentration of 1 µg/ml of progesterone in the organ bath. The other strip was pretreated for 45 minutes with propylene glycol (15 µl), acting as a control, to determine whether the contractile pattern altered over the time period in the absence of progesterone. Following the above period of pretreatment with progesterone or control solution relaxin was added in increasing doses until a complete inhibition of spontaneous activity was achieved and in the KCl-assay at least a 20 percent reduction in the tetanic contraction occurred.

10.4 RESULTS

Rat

Consistent results were obtained in 32 of the 33 oestrogen-primed non-pregnant rats. Porcine relaxin in doses as low as 10 ng/ml inhibited both spontaneous myometrial activity and KCl-induced contractility (Figure 22). However, when 1 µg/ml progesterone was added to the organ bath 45 minutes prior to the administration of relaxin, the subsequent inhibition of myometrial contractility by porcine relaxin was either greater or could be achieved at a lower dose (as low as 2.4 ng/ml) than when relaxin was added alone. In 20 non-pregnant rats studied, the mean response to relaxin was significantly greater in the muscle pretreated with progesterone for concentrations of relaxin $\geq$80 ng/ml (Figure 23).

The relaxant effect of porcine relaxin on early pregnant rat (days 10-14) myometrium was less than in the non-pregnant rat and doses of 40-500 ng/ml had to be given to produce a greater than 20 percent reduction in myometrial contractility in the KCl bioassay (Figure 22). In the rat with a unilateral 14 day gestation the same dose of relaxin 600 ng/ml produced a 7 percent reduction in contractiliy in the pregnant horn and a 25 percent reduction in the non-pregnant horn. The myometrium was most sensitive to relaxin on day 10 of pregnancy and less so on day 14.

Rat myometrial strips from days 10 and 14 of pregnancy pretreated with progesterone required less porcine relaxin to inhibit myometrial contractility. On day 10 of pregnancy the amount of relaxin required to produce a 20 percent reduction in myometrial contractility in the KCl bioassay was 2 ng/ml for the progesterone treated muscle and 75 ng/ml for the control muscle.

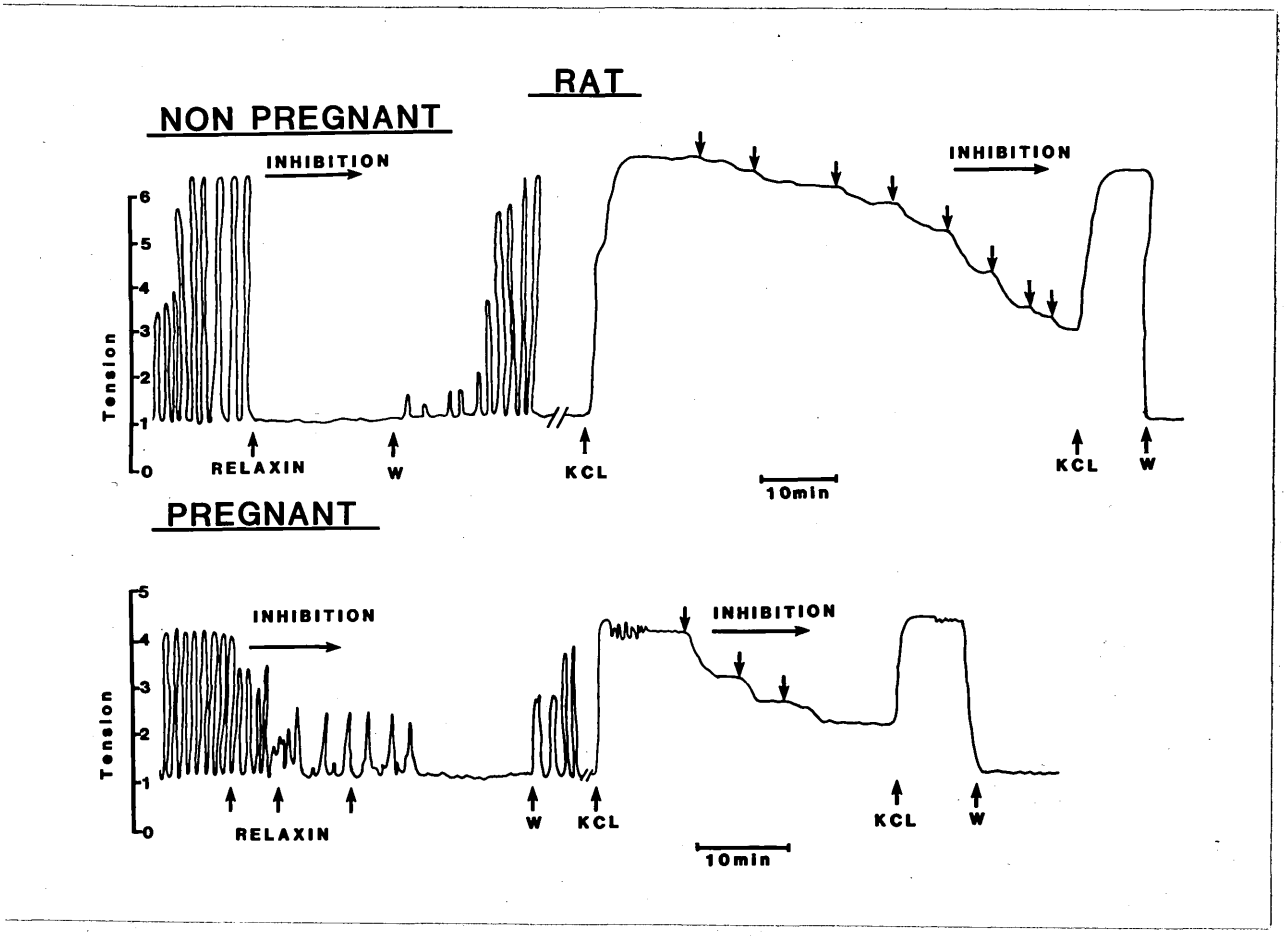


FIGURE 22 Myometrial contractility tracings from a non-pregnant and a pregnant rat. The first half of each tracing shows the inhibition of spontenaous contractility by pure porcine relaxin. The contractions returned after washing (w). The second half of each tracing shows the KCl induced tetanic contraction which can be proportionately reduced by adding increasing quantities of relaxin (arrows).

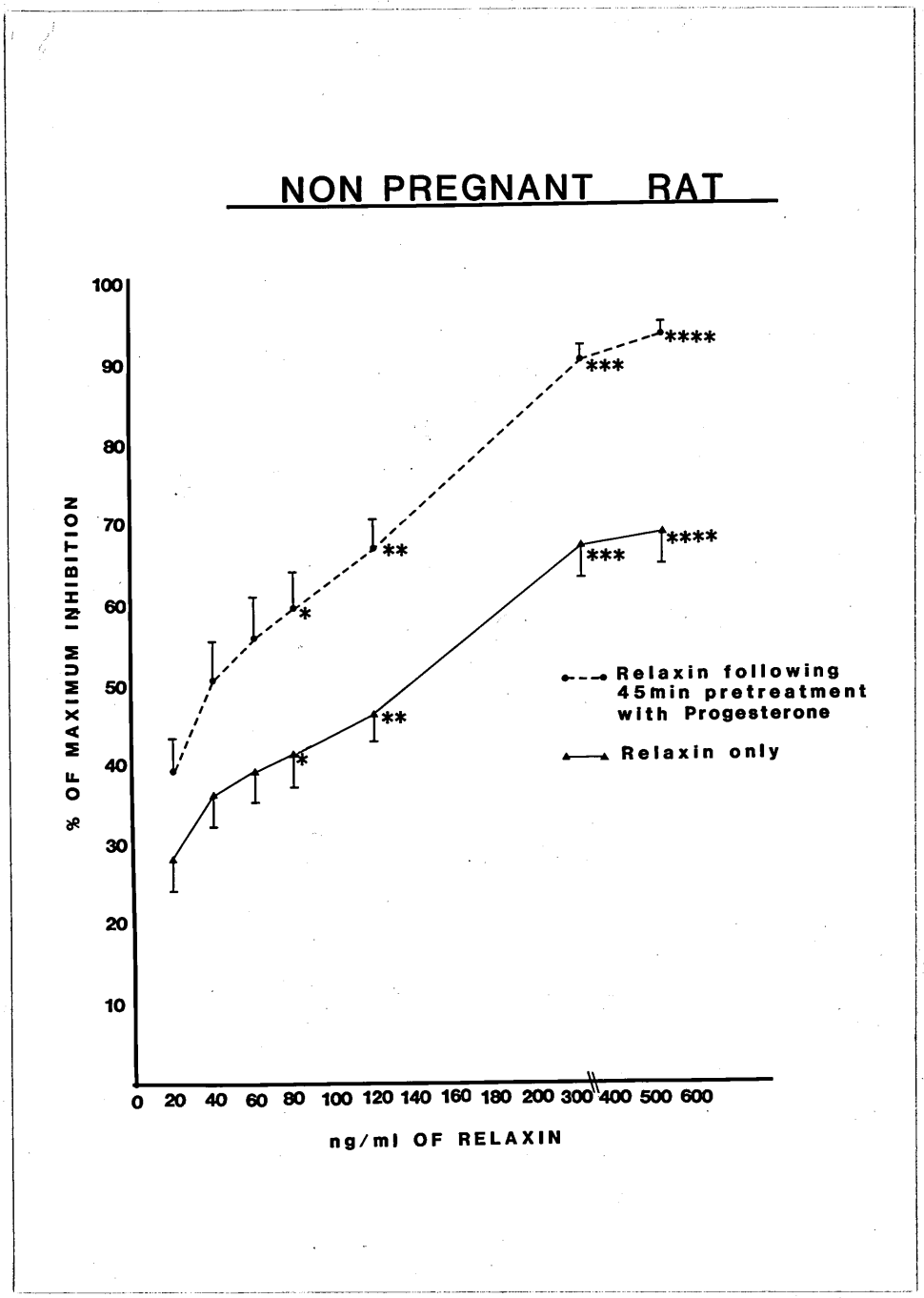


FIGURE 23 KCl bioassay comparing the response of non-pregnant rat myometrium to porcine relaxin when pretreated with progesterone to control samples from the same rat. The mean ± SEM is shown for 20 rats tested. *p<0.05, **p<0.02, ***p<0.005, ***p<0.001.

On day 14 of pregnancy rat myometrium was less sensitive to relaxin and the synergistic action of relaxin and progesterone was reduced, the mean amount of relaxin being required to produce a 20 percent reduction in myometrial contractility being 200 ng/ml in the progesterone treated muscle and 470 ng/ml in the control muscle.

By day 17 of pregnancy porcine relaxin had only a minimal effect on rat myometrium and by day 19 relaxin had no inhibitory effect on rat myometrium, either on spontaneous activity or in the KCl system for any of the relaxin doses tested (up to 15 µg/ml). Pretreatment with progesterone before the administration of relaxin also had no effect.

Pig

Porcine relaxin effectively inhibited spontaneous myometrial activity in all 7 of the non-pregnant pigs (Figure 24). The minimum effective doses of porcine relaxin to cause a 20 percent relaxation of the induced tetanic contraction of the non-pregnant pig myometrium ranged from 5-50 ng/ml.

Progesterone pretreatment of non-pregnant pig myometrium prior to the addition of porcine relaxin resulted in a greater percentage inhibition of the tetanic contraction in the KCl bio-assay (Figure 25). The mean effect of progesterone pretreatment was to approximately double the relaxant effect of porcine relaxin alone. The lowest effective relaxin dose on non-pregnant pig myometrium pretreated with progesterone ranged from 0.5-5 ng/ml.

Porcine relaxin was effective in inhibiting KCl-induced uterine contractions in all of the 23 pregnant pigs studied. Gestations studied ranged from 25-109 days and there was no

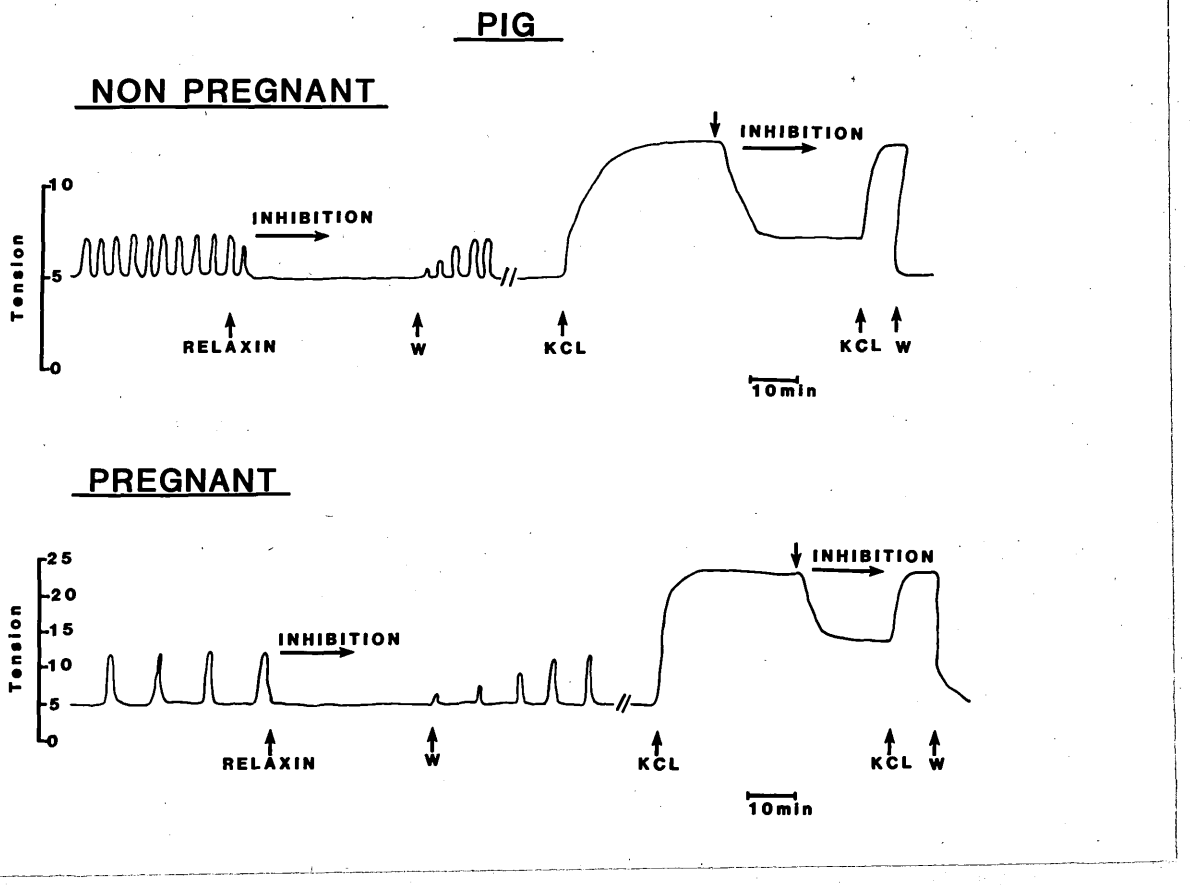


FIGURE 24 Myometrial contractility tracings from a non-pregnant and a pregnant sow. The first half of each tracing shows the inhibition of spontaneous contractility by pure porcine relaxin. The contractions return after washing (w). The second half of each tracing shows the KCl induced tetanic contraction which was relaxed by the addition of porcine relaxin to the organ bath (arrow).

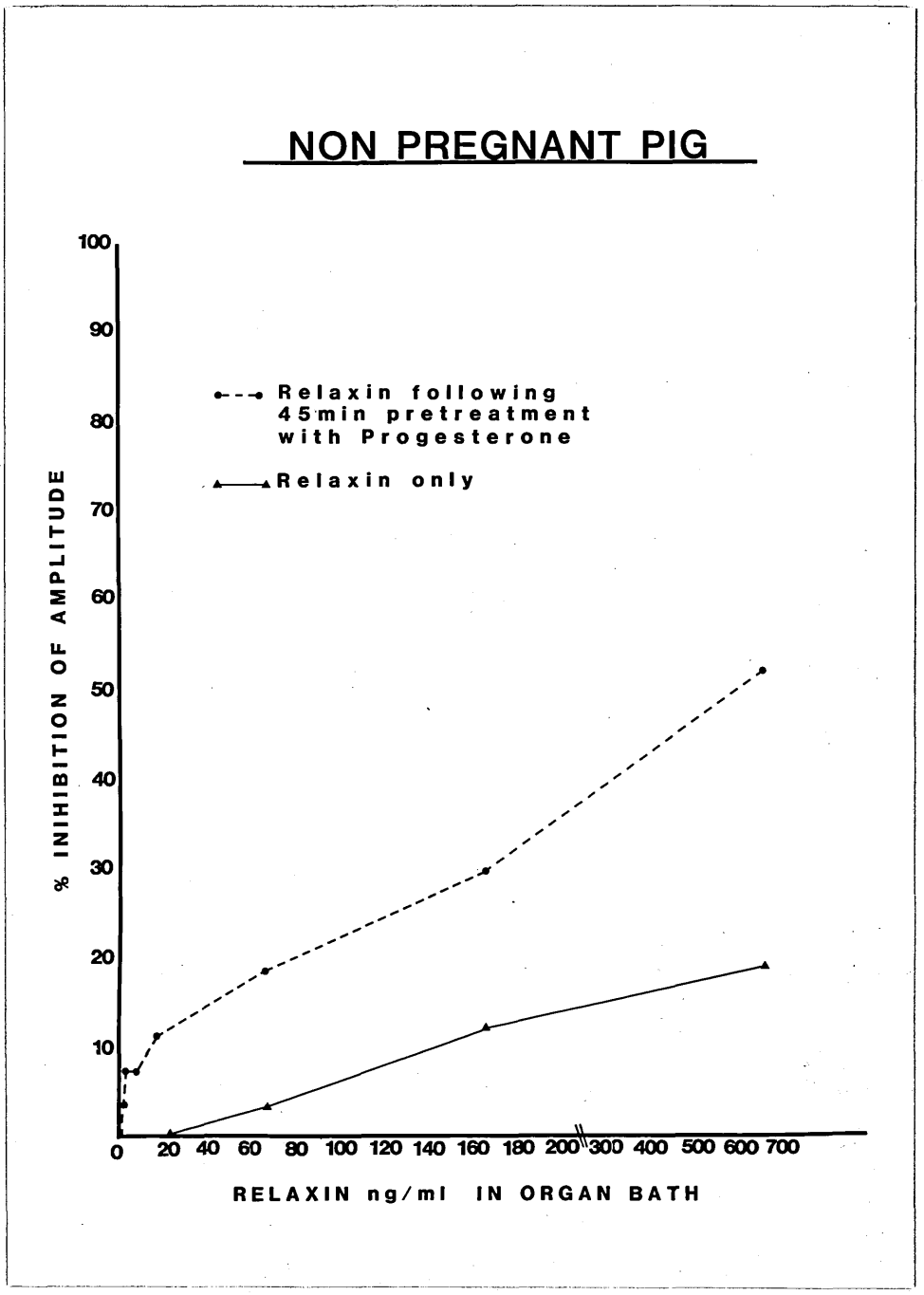


FIGURE 25 KCl bioassay showing approximately a doubling of the sensitivity of progesterone pretreated non-pregnant pig myometrium to increasing concentrations of porcine relaxin compared to a control strip of pig myometrium treated with porcine relaxin alone.

reduction in the myometrial sensitivity to relaxin during this period. Progesterone pretreatment of the myometrial strips again enhanced the effect of porcine relaxin. The lowest effective doses of porcine relaxin to inhibit pregnant pig myometrial activity ranged from 10-100 ng/ml in control specimens and 0.5-10 ng/ml in specimens pretreated with progesterone.

Human

Porcine relaxin in doses up to 10 µg/ml failed to inhibit spontaneous myometrial activity or induce relaxation in the KCl bioassay in 14 of the 17 non-pregnant specimens and 43 of the 47 myometrial strips obtained during pregnancy (Figure 26). Three non-pregnant and 3 pregnant human myometrial strips showed a minimal decrease in the amplitude of the spontaneous contractions following the administration of 3 µg porcine relaxin/ml (Figure 26). All the strips that showed a possible reaction to porcine relaxin were inner circular myometrial strips and the corresponding outer longitudinal muscle strips did not react to relaxin. Progesterone pretreatment had no effect on these or any of the other human specimens subsequently given relaxin.

Table 8 summarises the effect of pure porcine relaxin on gravid and non-gravid myometrium in the rat, pig and human. In all species tested where relaxin had an inhibitory effect on uterine contractility washing of the specimens brought back normal myometrial contractility. The progesterone dose and the propylene glycol used for pretreatment of the myometrial strips were insufficient to produce more than a 10 percent relaxation in the KCl bioassay.

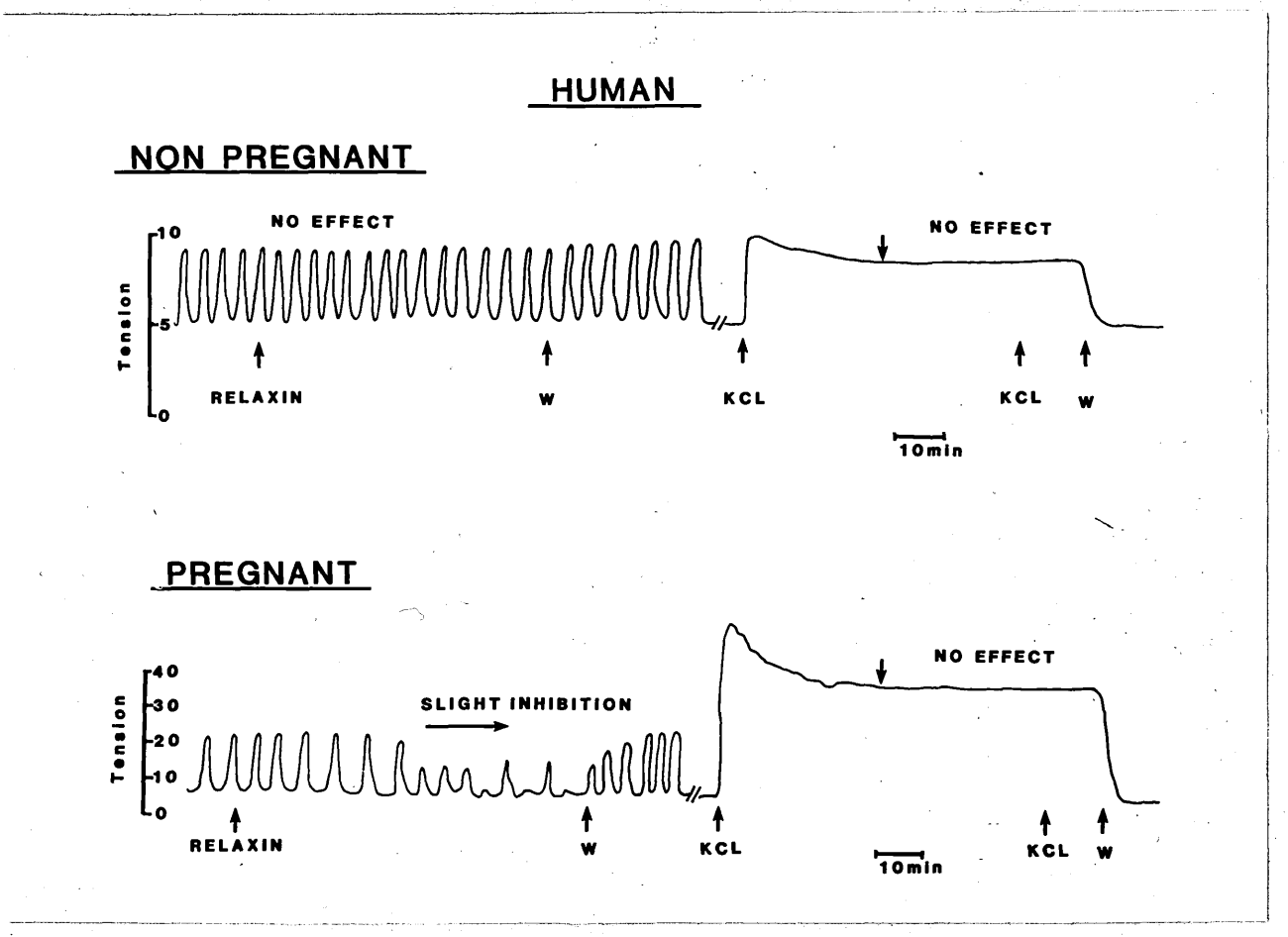


FIGURE 26 Myometrial contractility tracings from a non-pregnant and a pregnant human uterus. Porcine relaxin had no effect on spontaneous or KCl induced contractility in the human although on exceptional occasions as shown in the pregnant specimen slight inhibition of the amplitude of the spontaneous contractions did occur.

SUMMARY OF INHIBITION OF MYOMETRIAL CONTRACTILITY BY PORCINE RELAXIN

	RAT	PIG	HUMAN
NON PREGNANT	+	+	−
EARLY PREGNANCY	+	+	
MID PREGNANCY	+	+	−
LATE PREGNANCY	−	+	−

TABLE 8 Summary of the inhibitory effect of porcine relaxin on myometrial contractility in the non-pregnant and pregnant rat, pig and human. An inhibitory effect is represented as + and no effect as −. Early human pregnant myometrium has not been tested.

10.5 DISCUSSION

This study firstly confirms that pure porcine relaxin inhibits *in vitro* myometrial contractility in the oestrogen-primed non-pregnant rat and the pregnant rat except in late pregnancy. Rat myometrium appeared to be less sensitive to the effect of porcine relaxin as pregnancy progressed and was completely refractory to its effect by day 19 of pregnancy. The reason for the change in the myometrial response to porcine relaxin during pregnancy in the rat is not clear but may be due to a change in the status of the relaxin receptors in the myometrium. In a study by Mercado-Simmen et al (1982) the concentration of unoccupied relaxin receptors in rat myometrium decreased in late pregnancy in inverse relation to the increases in endogenous plasma relaxin at this time. The same study also showed that oestrogen enhances the binding activity of the rat uterus by increasing the concentration of myometrial relaxin receptors. High plasma levels of relaxin, on the other hand, diminished the sensitivity of continual relaxin exposure by markedly reducing the number of available myometrial receptors. The work of Mercado-Simmen et al (1982) suggested that the relaxin receptor concentration in rat myometrium is controlled through co-existing occupancy and down-regulation and by oestrogen through synthesis of new receptor sites. The possibility that other mechanisms and hormones might also modulate relaxin receptors was not excluded. Similar relaxin receptor studies have not yet been performed in the human.

This study also confirmed the recent report of Sarosi et al (1983) that porcine relaxin and progesterone are synergistic in inhibiting *in vitro* myometrial contractility in the non-pregnant rat. Similar synergism in early and mid pregnancy, as reported in this study, has not previously been described in the rat. Progesterone

treatment could not overcome the refractory state of late pregnant rat myometrium to porcine relaxin. The increased sensitivity of rat myometrium to relaxin after progesterone pretreatment significantly improves this method of bioassay of relaxin allowing much smaller amounts of relaxin or relaxin-like activity to be detected in biological fluids or tissue extracts during the monitoring of biological activity at the time of relaxin purification.

There has been no previous report in the literature of the in vitro effect of pure porcine relaxin on porcine myometrial contractility either in the non-pregnant or pregnant state. Such a study is clearly important as the relaxin in such an experiment is species specific. In the pig, porcine relaxin effectively inhibited myometrial contractility before and throughout the period of pregnancy tested (days 25-109), i.e. even in late pregnancy. Progesterone was again synergistic with relaxin in the pig with regards to its inhibitory effect on myometrial activity. The consistent effectiveness of this action of porcine relaxin on the pig was in marked contrast to its almost total lack of action in both non-pregnant and pregnant human myometrial strips whether pretreated with progesterone or not. This suggests that either porcine relaxin is not recognised by human myometrial relaxin receptors or that relaxin plays a different role in the human to that in the pig or rat and does not play a role in uterine quiescence, at least in this fashion.

The former argument is supported by the report of Hudson et al (1983) that there is only approximately a 50 percent homology between the amino acid structure of the first human relaxin that has been characterised and porcine relaxin. Extracts of human corpora lutea, whilst not claimed to be pure preparations of human relaxin, are reported to inhibit the amplitude of spontaneous contractions of human uterine strips in vitro (Beck et al 1982) suggesting that when species

specific relaxin is used relaxin plays a similar role in the human as in the pig with regards uterine quiescence. In the present study frequent washing of the human myometrial strips over several hours did not result in responsiveness to exogenous porcine relaxin suggesting that the lack of response was unlikely to be due to total occupancy of the relaxin receptors by endogenous human relaxin.

However, in contrast to the ineffectiveness of porcine relaxin on human myometrium in vitro, it has recently been reported that porcine relaxin inhibits human cervical smooth muscle activity in 18 percent of non-pregnant specimens, 68 percent of early pregnant and 100 percent of term pregnant specimens tested (Norström et al 1984). Clinical trials also show that vaginally applied pure porcine relaxin is effective in most patients at term in producing varying degrees of cervical softening and dilatation without apparent myometrial stimulation (MacLennan, Bryant-Greenwood & Greenwood 1983). Thus, relaxin receptors in the human cervix may differ from those in human myometrium and appear to be able to recognise porcine relaxin, especially near term. This suggests a mode of action and role for relaxin in the process of cervical ripening that is separate from its possible role in maintaining uterine quiescence earlier in pregnancy. The potential availability of genetically engineered human relaxin in the near future for similar in vitro trials should help clarify whether the differing species response to porcine relaxin is due to the specificity of human relaxin for human receptors. Access to human relaxin should also allow further study of the biological variations in the sensitivity of target organs to relaxin during pregnancy.

The mechanism of action of relaxin in inhibiting myometrial and cervical smooth muscle contractility in the human is not clearly established. In the rat, relaxin decreases the activity of myosin light-chain kinase resulting in a decrease in myosin phosphorylation

and actin-activated adenosine triphosphatase activity (Nishikori et al 1983). Enzymatic phorphorylation and dephosphorylation of the myosin light-chains are the key elements in the regulation of smooth muscle contractility. Activation of myosin light-chain kinase by its phosphorylation facilitates the temporary combination of actin and myosin into actomyosin which in turn produces a contractile state in smooth muscle. Myosin light-chain kinase activity is increased by calmodulin and adequate cellular calcium levels and decreased by cAMP-mediated phosphorylation of the kinase. A preliminary report (Weiss 1984) suggests that relaxin inhibits calmodulin activity. Relaxin also increases cAMP activity (Cheah & Sherwood 1980). Thus, relaxin influences two of the three main regulatory systems which control myosin light-chain kinase activity. The third regulator of myosin light-chain kinase activity is calcium, the intracellular levels of which are lowered by progesterone. Thus, the rate of myosin light-chain phosphorylation appears to be under the influence of both relaxin and progesterone. They both seem to be inhibiting the same intracellular mechanism in smooth muscle but their precise interaction and synergism at a biochemical level deserves more study.

CHAPTER 11

RIPENING OF THE HUMAN CERVIX AND INDUCTION OF LABOUR WITH PURIFIED PORCINE RELAXIN

11.1 SUMMARY

In a randomised double blind trial 10 of 30 patients given 2 mg of intravaginal purified porcine relaxin on the evening before surgical induction of labour went into labour before the proposed induction. Of 30 control patients, none went into labour. Of the 30 patients treated with relaxin, 25 had improved cervical scores after treatment and significantly fewer required agumentation in labour with intravenous oxytocin than the control group. Administration of relaxin was associated with very little increase in uterine activity and no side effects. The trial shows that exogenous relaxin causes cervical ripening and can initiate parturition. Endogenous relaxin may have a similar role.

11.2 INTRODUCTION

Relaxin is a polypeptide hormone, structurally related to insulin, first extracted and prepared in an impure form by Hisaw in 1926. It has been found in all species tested. In women it has been detected only in the corpus luteum of pregnancy (Weiss et al 1978). In many species, relaxin plays a major part in cervical ripening before parturition (Steinetz, O'Byrne & Kroc 1980). However, its role in human parturition has not been established. In most animals studied, serum relaxin levels are highest shortly before parturition. In women, serum levels of relaxin do not rise towards the end of pregnancy but cervical-tissue concentrations do rise (Von Maillot et al 1977). A rise in relaxin levels in early human labour has been described for the first time in Chapter 5. Early clinical studies with impure extracts of relaxin given parenterally or orally produced conflicting results but suggested overall that relaxin, or contaminants in the extract, produced cervical softening, and that in higher doses it inhibited uterine contractions (McCarthy, Ervine & Laufe 1957; Eichner et al 1958; Kupperman, Rosenberg & Cutler 1958).

Porcine relaxin can now be purified and its effects of the human cervix can be tested without the previous complication of possible active impurities in the ovarian extract. No clinical trial of purified relaxin in the human had yet been published prior to this paper. Recent work (Appendix 1) on prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and cervical ripening has shown that administration by the intravaginal route achieves direct and rapid absorption of this hormone to the site of action without systemic side effects. Thus, this route has been chosen for an investigation of the effect of purified relaxin on cervical ripening and parturition.

11.3 PATIENTS AND METHODS

Relaxin was obtained from the ovaries of pregnant sows and was purified by the method of Sherwood and O'Byrne (1974) with minor modifications as described by Kwok et al (1980). The relaxin used in this study had a biopotency of 1500 GPU/mg relative to the reference preparation NIH-R-PI (potency 442 GPU/mg). The purified relaxin used coincided with the CM-a' peak as described by Sherwood and O'Byrne (1974) in the characterisation and isolation of relaxin and was of the same homogeneity as the relaxin used for the analysis of the primary structure and sequence studies of porcine relaxin (James et al 1977).

Permission for the clinical trials was obtained from the research and ethics committee of The Queen Victoria Hospital, Adelaide. In a dosage trial 16 patients were given relaxin vaginally 15 h before surgical induction of labour, each patient receiving ½, 1, 2 or 4 mg of relaxin. The fetal heart, uterine activity, and clinical status of the patient were closely monitored. This preliminary trial suggested that both the 2 mg and 4 mg doses might be associated with a degree of cervical ripening without apparent side effects, and a 2 mg dose was chosen for the clinical trial now described.

A randomised double blind trial involving 60 patients was set up. Criteria for inclusion in the trial were: singleton pregnancy, cephalic presentation, unscarred uterus, maternal height over 150 cm, and informed consent from the patient. The patients were randomly allocated to receive either 2 mg relaxin diluted in 10 ml water or a placebo (10 ml sterile water), the allocated constituent being mixed with 700 mg tylose granules (Hoechst) to make a viscous gel. Close matching of the groups was apparent by the end of the trial in all respects except that the mean initial cervical score (modified Bishop score) (Calder, Embrey & Tait 1977) of the relaxin group was 1.6

VARIABLE	PLACEBO GROUP (n = 30)	RELAXIN GROUP (n = 30)
Mean maternal age (yr)	26.1	25.0
Mean gestation (wk)	40.0	40.4
Mean parity	0.6	0.6
Mean birth weight (kg)	3.4	3.4
Mean cervical score at priming	4.3	5.9

TABLE 9 Comparison of treatment and control groups

points higher than that of the placebo group (Table 9).

A.H.M., unaware of the treatment allocation, assessed the cervical score at 5 p.m. on the evening before proposed induction of labour. The gel was then placed in the posterior vaginal fornix, by means of a soft latex catheter and a syringe. No attempt in this trial was made to stretch the cervix or sweep the membranes. The patient was asked to remain in bed for an hour. Next morning at 8 a.m. the cervical score was reassessed by A.H.M. Thereafter, the labour was managed by obstetricians unaware of the constituents of the vaginal gel.

Statistical analysis of the results was done with Student's (one-tailed) t test, Fisher's exact test, and the χ^2 test.

11.4 RESULTS

The effect of the placebo gel and the relaxin 15 h after vaginal application is shown in Table 10. Labour became established in 10 of the 30 patients receiving relaxin, whereas no patient in the control group went into labour ($p<0.0005$, Fisher's exact test). The mean improvement in the cervical score was 3.38 for the relaxin group and 0.86 for the placebo group ($p<0.0025$, Student's t test). The improvement in the cervical score of the relaxin group was still significant even among patients who did not go into labour ($p<0.0025$, Student's t test). The cervical score remained unchanged in only 5 patients receiving relaxin, whereas 18 patients receiving placebo showed no evidence of improvement ($p<0.0005, \chi^2$).

Significantly fewer women in the group receiving relaxin needed augmentation of labour with intravenous oxytocin ($p<0.005$, χ^2 test) (Table 11). The relaxin group also had shorter labours ($p<0.05$, χ^2 test). However, there was no significant difference between the two groups in mode of delivery, analgesic requirements, or Apgar score of the infant at delivery.

The cervical score in the relaxin group improved regardless of the initial cervical score (Table 12), parity (Table 13), or gestation (Table 14). Eight of the 10 patients who laboured had a favourable cervical score (7-9), but there was no apparent relationship between parity or gestation and the onset of labour after treatment with relaxin.

No side effects were experienced by the patients receiving relaxin and no neonatal complications were recorded. Although mild uterine activity was sometimes noted electronically by means of an abdominal pressure transducer after vaginal examination and application of the relaxin, only those who laboured were aware of a change

in their previous pattern of uterine activity. In fact, where labour did not supervene it was not possible clinically to detect any difference between the two groups. No abnormal fetal heart pattern was recorded after application of the gel and before labour started. Two babies from the relaxin group and 4 from the placebo group showed signs of mild respiratory depression at birth and required active resuscitation. One patient in the relaxin group had a caesarean section for cephalo-pelvic disporportion. There were 3 caesarean sections in the placebo group, the indications being cervical dystocia, failure to progress, and fetal distress.

OUTCOME	PLACEBO GROUP (n = 30)	RELAXIN GROUP (n = 30)
Delivered at 15 h	0	7
Established in labour	0	3
Average change in cervical score	0.86	3.38
Average change where labour did not supervene	0.86	2.08
No. with no change	18	5

TABLE 10 Outcome on morning after treatment of cervix

OUTCOME	PLACEBO GROUP (n = 30)	RELAXIN GROUP (n = 30)
No. augmented with oxytocin	23	13
Length of labour (hr)* (mean ± SD)	8.2 (± 3.2)	6.6 (± 3.1)
Caesarean section	3	1
Instrumental delivery	15	14
Pethidine given (mean dose)	13 (115 mg)	15 (100 mg)
Epidural block (mean dose)	15 (31 ml)	12 (23 ml)
No analgesia in labour	4	8
Apgar score at 1 min.	8.1	8.1
Apgar score at 5 min.	9.0	9.0

TABLE 11 Outcome of labour in control and relaxin treated groups

*Excluding Caesarean section

INITIAL SCORE	MEAN IMPROVEMENT IN CERVICAL SCORE	
	PLACEBO	RELAXIN
0 - 3	0.9 (12)	2.8 (6)
4 - 6	0.9 (14)	2.8 (9)
7 - 9	0.5 (4)	4.0 (15)

TABLE 12 Initial cervical score and mean change in cervical score 15 h after treatment.

No. of patients in parentheses

PARITY	MEAN IMPROVEMENT IN CERVICAL SCORE	
	PLACEBO	RELAXIN
0	0.9 (16)	2.8 (16)
1	0.7 (11)	4.3 (10)
2+	1.3 (3)	3.9 (4)

TABLE 13 Parity and mean change in cervical score 15 h after treatment.

No. of patients in parentheses

GESTATION (wk)	MEAN IMPROVEMENT IN CERVICAL SCORE	
	PLACEBO	RELAXIN
38 - 39	0.8 (13)	2.2 (6)
40 - 41	1.0 (12)	3.7 (17)
41+	0.6 (5)	3.7 (7)

TABLE 14 Gestation and mean change in cervical score 15 h after treatment.

No. of patients in parentheses

11.5 DISCUSSION

The placebo and treatment groups were well matched except that the mean cervical score of the relaxin group was 1.6 points higher than that of the placebo group before treatment. In particular, more patients in the relaxin group had a favourable cervical score (Table 12) and 8 of the 10 patients who went into labour were in this group. Thus, the apparent effectiveness of relaxin may have been biased by this chance difference between the two groups. However, in a similar trial (Appendix 1) of cervical ripening with $PGF_{2\alpha}$, there were 11 patients in the placebo group with an initial cervical score between 7 and 9. However, only 1 of these 11 went into labour, compared with 8 of 15 patients with a similar cervical score in the relaxin group. A statistically significant improvement in the cervical score was seen in the 20 patients treated with relaxin who did not labour and an improvement in the mean cervical score was noted whatever the initial score before treatment (Table 12). Thus the difference in the mean initial cervical scores between the two groups is unlikely to account for the effect of relaxin on cervical ripening and the initiation of parturition. However, the difference in the initial cervical score may account to some extent for the shorter labours in the relaxin group.

In a double blind dose trial (Appendix 2) which was identically structured to this trial, 25 mg of intravaginal $PGF_{2\alpha}$ was associated with a mean improvement in the cervical score of 3.76, and labour started in 9 of the 30 patients treated. A higher dose (50 mg) of intravaginal $PGF_{2\alpha}$ improved the cervical score by 4.63, and 10 of the 30 patients went into labour. Thus, the effect of 2 mg of intravaginal relaxin is comparable to these doses of intravaginal $PGF_{2\alpha}$.

The relationship between relaxin and prostaglandin in women is

unknown, but both are probably necessary for cervical ripening. Injections of $PGF_2\alpha$ in pregnant pigs on day 112 caused sharp rises in plasma relaxin concentrations (Sherwood et al 1976). However, in 7 patients given intravenous $PGF_2\alpha$ to induce labour a statistically significant rise in serum relaxin levels was not seen (Hochman et al 1978). A prolonged rise in plasma relaxin in the pregnant sow after oestrogen administration (F. Amato, G. Warnes, M. Ralph, G.D. Bryant-Greenwood, F.C. Greenwood & R.F. Seamark, unpublished) and the postulated relationship between oestrogen and prostaglandin suggests that the triad of hormones - oestrogen, prostaglandin and relaxin - requires study in man.

This trial suggests that endogenous relaxin might play an important part in the ripening of the human cervix and in the initiation of parturition. The mode of action of endogenous or exogenous relaxin is not clear. In this trial, unless labour supervened, the patient was not aware of any uterine activity after application of the relaxin. However, in some patients increased uterine activity was noted electronically by means of an abdominal pressure transducer, though this may have been due to a transient rise in endogenous prostaglandin levels caused by the vaginal examinations. Whether the relaxin caused cervical ripening by indirect stimulation of uterine contractions or by a direct effect on the cervix is not known. Because the contractions were small and inconsistent, a direct effect seems more likely, and attempts are in progress to characterise relaxin receptors in the cervix.

The effects of intravaginal relaxin in women are similar to those of intramuscular relaxin given to pregnant luteectomised sows, in which premature cervical dilatation, premature labour and a reduction in the length of labour resulted (Kertiles & Anderson 1979). Lactation in those sows was severely reduced. However, almost all mothers

in this trial breast-fed, and no reduction in lactation was detected.

Two mg relaxin appears to be at least as safe and effective as 25 mg of intravaginal $PGF_2\alpha$, and in this trial labour was significantly shorter than in the control group. Further dose studies and an increased availability of porcine or human relaxin may eventually make it a useful alternative in the induction and management of labour. Meanwhile, the relationships between oestrogen, prostaglandin, and relaxin in their effects on cervical ripening and parturition may be studied empirically (Chapter 12).

The role of endogenous relaxin in human parturition needs further investigation. In this study exogenous purified porcine relaxin induced cervical changes in most patients and in some was associated with the initiation of parturition. If there are receptor sites for relaxin in the human cervix in late pregnancy, a role for the endogenous hormone would be suggested perhaps by a local action not reflected in significant changes in serum relaxin.

CHAPTER 12

CERVICAL RIPENING WITH COMBINATIONS OF VAGINAL PROSTAGLANDIN $F_2\alpha$, OESTRADIOL AND RELAXIN.

12.1 SUMMARY

The first of a two part trial consisted of a double blind randomised pilot study in which 4 groups of 10 patients near term received one of the following hormonal combinations in a vaginal gel 15 h before surgical induction of labour: (1) prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and relaxin, (2) relaxin and oestradiol, (3) oestradiol and $PGF_2\alpha$, and (4) relaxin, oestradiol and $PGF_2\alpha$. In each group the mean cervical score improved after treatment; the relaxin/$PGF_2\alpha$ combination was associated with the greatest improvement in cervical score (4.8). The highest incidence of subsequent labour was also seen in the relaxin/$PGF_2\alpha$ group (40%). However, with the exception of the latter group, the clinical effects of these hormonal combinations were neither greater nor smaller than the previously published effects of these hormones used individually in similar circumstances. The second part of the study further explored the possibility of an additive effect of relaxin and $PGF_2\alpha$ in combination as suggested by the pilot study, and an additional 40 patients were given this combination. Analysis of these larger numbers showed no additive effect when these hormones were used in combination compared with when they were used individually. Thus, in the circumstances described, there is no clinical advantage to the concurrent administration of any combination of relaxin, $PGF_2\alpha$, and oestradiol with regard to cervical ripening and/or the initiation of parturition.

12.2 INTRODUCTION

Intravaginal prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (MacLennan & Green 1979 (Appendix 1)), oestradiol (Gordon & Calder 1977) and purified porcine relaxin (MacLennan et al 1980 (Chapter 11)) have been used individually to ripen the cervix and, with variable success, to initiate labour. No clinical trials have been reported of the effects of combinations of hormonal cervical ripening agents. The present trial was designed to examine empirically whether there is any clinical advantage to the use of these hormones in combination and to study any apparent relationships between their modes of action when given in these circumstances.

12.3 PATIENTS AND METHODS

Relaxin was obtained from the ovaries of pregnant sows and was purified by the method of Sherwood and O'Byrne (1974) with minor modifications as described by Kwok et al (1980). The relaxin used in this study had a biopotency of 1500 guinea-pig units (GPU) /mg relative to the reference preparation NIH-R-PI (potency 442 GPU/mg). The purified relaxin used was equivalent to the CM-a' peak described by Sherwood and O'Byrne (1974) and is the same as that used for the analysis of the primary structure and sequence studies of porcine relaxin (James et al 1977).

Permission for the clinical trials was obtained from the research and ethics committee of The Queen Victoria Hospital, Adelaide. As only a limited supply (140 mg) of relaxin was available, a preliminary double blind randomised pilot study was set up involving 4 groups of 10 patients. The patients in each group received 2 mg relaxin and 10 mg oestradiol-17β; 10 mg oestradiol-17β and 25 mg $PGF_2\alpha$; 2 mg relaxin and 25 mg $PGF_2\alpha$; or 2 mg relaxin, 25 mg $PGF_2\alpha$ and 10 mg oestradiol-17β. It was proposed that the remaining 80 mg relaxin be used to examine in greater numbers the combination that appeared most effective. Thus, at the end of the double blind randomised study, an additional 40 patients were given the combination containing 2 mg relaxin and 25 mg $PGF_2\alpha$. Close matching of the 4 groups in the initial randomised trial was apparent by the end of the study (Table 15). The patients from similar pretreatment trials (Chapter 11 and Appendix 2), shown for comparison in Table 16, also had clinical characteristics comparable to those of the overall total of 50 patients given the relaxin/$PGF_2\alpha$ combination.

Criteria for inclusion in the study were singleton pregnancy, cephalic presentation, unscarred uterus, maternal height over 150 cm,

VARIABLE	OESTRADIOL + RELAXIN (n = 10)	OESTRADIOL + $PGF_{2\alpha}$ (n = 10)	RELAXIN + $PGF_{2\alpha}$ (n = 10)	RELAXIN + $PGF_{2\alpha}$ + OESTRADIOL (n = 10)
Mean maternal age (yr)	26.0	25.8	26.3	27.1
Mean gestation (wk)	40.6	40.4	40.1	39.6
Mean parity	1.2	1.0	0.6	1.4
Mean birth weight (g)	3500	3500	3500	3500
Mean cervical score at priming	4.2	3.9	4.5	5.1

TABLE 15 Comparison of treatment groups in preliminary trial

no history of asthma, and informed patient consent. The hormonal combinations were made up to 10 ml with distilled water and mixed with 700 mg of water-soluble cellulose (Tylose) granules (Hoechst) to make a viscous gel. One of the authors (AHM), unaware of the treatment allocation, assessed the cervical score (modified Bishop score) (Calder, Embrey & Tait 1977) at 5.00 p.m. on the evening before the proposed induction of labour. The gel was then placed in the posterior vaginal fornix by means of a soft latex catheter and a syringe. No attempt was made in this trial to stretch the cervix or sweep the fetal membranes. The patient was asked to remain in bed for an hour. The next morning at 8.00 a.m. the cervical score was reassessed by the same author. Thereafter, the labour was managed by obstetricians unaware of the constituents of the vaginal gel. Statistical analysis of the results was done with the Student one-tailed t test, the Fisher exact test and the χ^2 test.

12.4 RESULTS

The outcome of the pilot study is shown in Table 17. The mean cervical score improved after treatment in each group, the greatest improvement occurring in the group receiving the relaxin/$PGF_2\alpha$ combination. This combination was also associated with the highest incidence of labour. However, the differences between the results from each group were not statistically significant for the numbers studied.

As the relaxin/$PGF_2\alpha$ combination was associated with the greatest clinical effect in the pilot trial, an additional 40 patients were given this combination to examine more thoroughly any enhancement of these hormones' individual actions. The results of all 50 patients given the relaxin/$PGF_2\alpha$ combination are shown in Table 16, in which they are compared with previously reported trials (Chapter 11 and Appendix 2) of cervical pretreatment with a placebo, 2 mg relaxin, or 25 mg $PGF_2\alpha$ alone. The combination of relaxin and $PGF_2\alpha$ given to 50 patients was not associated with a greater mean improvement in cervical score or a higher incidence of labour than pretreatment with either relaxin or $PGF_2\alpha$ alone.

There was no statistical difference in the outcome of labour between any of the treatment groups in this trial with respect to length of labour, analgesic requirements, mode of delivery, or Apgar scores. No maternal or neonatal side effects were detected during the study.

OUTCOME	OESTRADIOL + RELAXIN (n = 10)	OESTRADIOL + $PGF_2\alpha$ (n = 10)	RELAXIN + $PGF_2\alpha$ (n = 10)	RELAXIN + $PGF_2\alpha$ + OESTRADIOL (n = 10)
No. in established labour or delivered at 15 h	1	3	4	1
Mean improvement in cervical score	2.3	4.0	4.8	3.0
Mean improvement where labour did not supervene	1.8	1.9	2.8	2.3
No. in whom no cervical ripening	3	2	1	4
Mean length of labour (hr)	6.8	8.1	7.1	7.3

TABLE 17 Outcome of cervical pretreatment on the morning of proposed surgical induction

OUTCOME	CONSTITUENTS OF VAGINAL GEL			
	PLACEBO (n = 30)	$PGF_{2\alpha}$ (25 mg) (n = 30)	RELAXIN (2 mg) (n = 30)	$PGF_{2\alpha}$ (25 mg) + RELAXIN (2 mg) (n = 50)
No. in established labour or delivered at 15 h	0	9	10	17
Mean improvement in cervical score	0.9	3.8	3.4	3.7
Mean change where labour did not supervene	0.9	1.9	2.1	2.0
No. in whom no cervical ripening	18	6	5	7
Mean length of labour (hr)	8.2	7.8	6.6	7.3

TABLE 16 Outcome of pretreatment with relaxin and prostaglandin $F_{2\alpha}$ given individually and in combination.

12.5 DISCUSSION

The pilot study suggested no major enhancement of the individual effects (Gordon & Calder 1977; MacLennan et al 1980; MacLennan & Green 1980) of these hormones when used in combination, with the possible exception of relaxin and $PGF_{2\alpha}$. The limited quantity of purified relaxin available for this trial curtailed the study of large numbers in each group in the pilot study. The study of the influence of exogenous hormones on parturition in patients close to their expected date of delivery is open to error when the number of patients studied is small. There can be biological variation in the response to these hormones when they are given via this route (MacLennan & Green 1979; Gordon & Calder 1977; MacLennan et al 1980), and there is a small chance of spontaneous labour in any 15 h period near term (Control Group, Appendix 1). The empiricism of the model is further compounded by the lack of information on different rates of absorption of the different classes of hormones, prostaglandins, steroids and peptides, from the gel by the tissues, such that absorption may be sequential. It was important to study the relaxin/$PGF_{2\alpha}$ combination in greater numbers to allow better analysis of the possibility of an additive or synergistic effect of these hormones in combination. After treatment of 50 patients with relaxin and $PGF_{2\alpha}$ in combination, it was clear that there was no such enhancement of these hormones' individual effects. However, future administration of these hormones in a known time sequence, rather than in apparent combination, may be more physiologic if, for example, prior oestrogen or prostaglandins stimulate an increase in relaxin receptor sites. Oestrogen administration has been shown to induce relaxin receptors in the uterus of ovariectomized rats but not in the cervices of ovariectomized, oestrogen treated sows (unpublished observations). In the rat, prost-

aglandin synthetase inhibitors block the cervical ripening effect of relaxin, suggesting that prostaglandins may mediate the effect of relaxin on the cervix (Kennedy 1976). However, in this study $PGF_2\alpha$ did not enhance the effect of relaxin when given at the same time as relaxin, and therefore, if $PGF_2\alpha$ does mediate the response of the human cervix to relaxin, it may require administration before the treatment with exogenous relaxin.

The 3 hormones studied did not induce separate or complimentary detectable cervical changes when given in combination. The possibility that they may act in sequence through the same pathway to produce the same effect remains to be examined. Thus, there would appear to be no clinical advantage to using these hormones in combination in the circumstances described, but their future use in sequence might prove more effective and give further insight into their relationships at the onset of parturition.

CHAPTER 13

RIPENING OF THE HUMAN CERVIX AND INDUCTION OF LABOUR WITH INTRACERVICAL PURIFIED PORCINE RELAXIN

13.1 SUMMARY

In a randomised double blind placebo controlled trial involving 54 patients, a viscous gel containing either distilled water or 1 or 2 mg pure porcine relaxin was instilled in the cervical canal on the evening prior to the surgical induction of labour. Seven of the 36 patients receiving relaxin laboured overnight. Of the control patients none went into labour. Both the 1 mg dose and the 2 mg dose significantly improved the mean cervical scores compared to the placebo treatment. The favourability of the cervix at the time of the initial treatment did not influence the effect of the 2 mg dose but when the results were analysed by parity the 2 mg dose was more effective in the primiparous patients and the 1 mg dose was more effective in the multiparous patients. Compared to the results of previous trials the intracervical application of porcine relaxin is no more effective than its intravaginal application in effecting cervical ripening or inducing labour. This trial confirms the responsiveness of the human term cervix to exogenous relaxin and supports the suggestion that endogenous relaxin may play a similar role in facilitating cervical ripening and parturition.

13.2 INTRODUCTION

In most species studied there is a surge in serum relaxin approximately 24 h prior to the onset of parturition. In association with this increase in relaxin there is a shortening, softening and opening of the cervix which is known as cervical ripening. It is postulated that in the human one of the main roles of the polypeptide hormone relaxin is to facilitate connective tissue remodelling during reproduction such as occurs during the cervical ripening process (Chapter 1). Human relaxin is not yet available for *in vitro* or *in vivo* trials but porcine relaxin has recently been purified in sufficient quantities for clinical trials. To date, there have been three clinical trials with pure porcine relaxin (MacLennan et al 1980 (Chapter 11); MacLennan et al 1981 (Chapter 12); Evans et al 1983). The first two showed that 2 mg of vaginally applied porcine relaxin in a tylose gel promoted cervical ripening in over 80 percent of patients near term and apparently initiated labour in approximately one-third of the treatment groups. The third trial confirmed the efficiency of porcine relaxin as a cervical ripening agent when applied to the cervix in Carbowax pessaries containing either 2 or 4 mg of relaxin. Vaginally applied prostaglandins and relaxin appear to work in sequence to produce the same clinical (Chapter 12) and histological (Chapter 14) changes in the cervix. When given intracervically rather than vaginally prostaglandin-containing gels appear to be more effective as cervical ripening agents and can be given in smaller doses to induce labour (Wingerup, Anderson & Ulmsten 1979; Ekman et al 1983).

This study examines for the first time the efficacy of pure porcine relaxin as a cervical ripening agent when applied intracervically in a viscous gel in two differing doses.

13.3 PATIENTS AND METHODS

Permission for the clinical trials was obtained from the research and ethics committee of The Queen Victoria Hospital, Adelaide and from the Australian Department of Health. A randomised placebo controlled double blind trial was designed involving 54 patients who were scheduled for surgical induction near term. Criteria for inclusion in the trial were singleton pregnancy, cephalic presentation, unscarred uterus, maternal height over 150 cm, a pretreatment cervical score of 6 or less (modified Bishop score) (Calder, Embrey & Tait 1977) and informed patient consent. The patients were randomly allocated to receive either a placebo (2 ml sterile water), 1 mg or 2 mg of purified porcine relaxin, the allocated constituent being mixed with 150 mg tylose granules (Hoechst) to make a viscous gel. Close matching of the groups was apparent by the end of the trial (Table 18). The mean parity of the placebo group was higher than the treatment groups but this did not reach statistical significance.

Relaxin was obtained from the ovaries of pregnant sows and was purified by the method of Sherwood and O'Byrne (1974) with the modifications of Walsh and Niall (1980). The relaxin used in this study had a biopotency of 1500 guinea-pig units (GPU) /mg relative to the reference prepapartion NIH-R-PI (potency 442 GPU/mg). The purified relaxin used was equivalent to the CM-a' peak described by Sherwood and O'Byrne (1974) and is the same as that used for the analysis of the primary structure and sequence studies of porcine relaxin (James et al 1977).

A.H.M., unaware of the treatment allocation, assessed the cervical score at 5 p.m. on the evening before the proposed induction of labour. The gel was then applied from the top of the cervical canal by means of a thin plastic cannula which was gradually withdrawn

VARIABLE	PLACEBO (n = 18)	1 mg RELAXIN (n = 18)	2 mg RELAXIN (n = 18)
Mean maternal age (yr)	25.1	28.4	25.8
Mean gestation (wk)	40.0	39.7	39.9
Mean parity	1.1	0.8	0.6
Mean birth weight (kg)	3.6	3.3	3.4
Mean cervical score at priming	3.7	3.7	3.4

TABLE 18 Comparison of treatment and control groups in the intracervical relaxin pretreatment trial

so that the 2 ml of gel was completely expelled into the cervical canal before removal of the cannula from the external os. No attempt was made in this trial to stretch the cervix or sweep the membranes. The patient was asked to remain in bed for 1 h. Next morning at 8 a.m. the cervical score was reassessed by A.H.M. Thereafter, the labour was managed by obstetricians unaware of the constituents of the vaginal gel.

Statistical analysis of the results was performed using Student's (one tailed) t test and Fisher's exact test.

13.4 RESULTS

The effect of the placebo gel and the relaxin gels 15 h after intracervical application is shown in Table 19. No patients in the placebo group laboured overnight but 7 of the 36 patients receiving relaxin became established in labour during this time ($p<0.05$, Fisher's exact test). One patient in each of the relaxin treated groups had spontaneous rupture of the membranes overnight in association with the onset of labour. The mean improvement in the cervical score was 1.1 for the placebo group. Compared to this group the mean improvement of 2.2 in the cervical score of patients treated with 1 mg relaxin was significant ($p<0.05$, Student's t test) as was the mean improvement of 3.0 following treatment with 2 mg relaxin ($p<0.025$). The difference in the results between the 1 and 2 mg groups was not statistically significant. The cervical score improved regardless of the initial cervical score in the 2 mg relaxin treated groups ($p<0.05$, Student's t test) but the improvement seen in the 1 mg relaxin treated groups as analysed by the initial cervical score did not reach statistical significance for the numbers studied (Table 20). When analysed by parity a statistically significant improvement in the cervical score was seen only in the primiparous patients receiving 2 mg relaxin and the multiparous patients receiving 1 mg relaxin ($p<0.05$, Student's t test) (Table 21). There was no statistical difference in the outcome of the labours (Table 22). No apparent side effects to the relaxin treatment were detected in the mothers or the babies and the indication given for each of the 5 caesarean sections was "failure to progress".

OUTCOME	PLACEBO (n = 18)	1 mg RELAXIN (n = 18)	2 mg RELAXIN (n = 18)
Delivered at 15 h	0	1	0
Established in labour	0	2	4
Mean change in cervical score	1.1	2.2	3.0
No. with no change	7	5	4

TABLE 19 Outcome of pretreatment with intracervical placebo or relaxin.

INITIAL SCORE	MEAN IMPROVEMENT IN CERVICAL SCORE		
	PLACEBO	1 mg RELAXIN	2 mg RELAXIN
0 - 3	1.2 (7)	2.9 (9)	3.4 (8)
4 - 6	1.1 (11)	1.7 (9)	2.7 (10)

TABLE 20 Analysis of results of intracervical relaxin pretreatment by initial cervical score.

No. of patients in parentheses

PARITY	MEAN IMPROVEMENT IN CERVICAL SCORE		
	PLACEBO	1 mg RELAXIN	2 mg RELAXIN
Primiparous	0.7 (6)	1.6 (11)	3.8 (11)
Multiparous	1.4 (12)	3.3 (7)	1.7 (7)

TABLE 21 Analysis of results of intracervical pretreatment by parity

No. of patients in parentheses

OUTCOME	PLACEBO (n = 18)	1 mg RELAXIN (n = 18)	2 mg RELAXIN (n = 18)
Augmented with oxytocin	14	13	12
Mean length of labour (hr)	7.1	6.2	7.3
Caesarean section	1	2	2
Instrumental delivery	2	3	3
Mean Apgar score at 1 min.	7.6	7.9	7.5
Mean Apgar score at 5 min.	8.8	9.1	9.1

TABLE 22. Outcome of labour in intracervical pretreatment trial

13.5 DISCUSSION

The results of this trial confirm the findings of the first clinical trials using purified porcine relaxin in showing that exogenous porcine relaxin has a cervical ripening effect on the human cervix near term. In the first published randomised double blind trial (MacLennan et al 1980) the relaxin treated group had a slight bias towards being more favourable at the time of the application of the gel. In this trial the placebo and treatment groups were well matched although the placebo group had a higher but not statistically significant different mean parity. There were 12 multiparous patients in the placebo group but only 7 in each of the treatment groups. This may account for the 7.1 h mean length of labour in the placebo group with is 1-2 h shorter than comparable placebo groups of lower mean parity in the other trials (Chapters 11 & 12) and, thus, explain why, unlike the previous trials, a significant reduction in the length of labour was not apparent in the relaxin treated group compared to the control group. The mean length of labour of 6.7 h in the relaxin treated groups is similar to the significantly reduced mean length of labour (6.6 h) of relaxin treated patients in the previous trials described in this thesis.

The improvement in cervical score and the incidence of subsequent labour in this trial of intracervical relaxin gel are no better than the results obtained following the intravaginal application of relaxin (Chapter 11). Although both methods were not compared in the same trial the similarity of the trials and the close matching of the patients studied in both trials suggests that there is no advantage in the intracervical application of relaxin compared to its vaginal application in a viscous gel. In the first trial (Chapter 11), 2 mg of purified porcine relaxin given intravaginally in tylose gel gave a

mean improved cervical score of 3.4 points and 33 percent of the patients laboured during the next 15 h. In this trial of intracervically applied relaxin the 2 mg group improved its mean cervical score 3.0 points and 20 percent of the patients laboured.

In this trial, only 2 mg of intracervical relaxin proved to be statistically better than the placebo in producing cervical ripening in the primiparous patients and 1 mg of relaxin was the only statistically significant effective dose in the multiparous patients. The favourability of the cervix at the time of the application did not appear to inhibit or enhance the action of the exogenous relaxin's cervical ripening effect. Evans et al (1983) did not analyse their patients by parity or initial cervical favourability but their results suggested that 2 mg of vaginally applied porcine relaxin was more effective than 4 mg. The greater efficacy of the smaller dose could not be explained although a down-regulation effect of "overdosing" was hypothesised. The same phenomenon may have occurred in the multiparous patients in this trial.

It is likely that at intracervical application of the gel some of the gel may have entered the neighbouring extraamniotic space and would have been in close contact with the fetal membranes. It is of interest to note that over the next 15 h only 2 out of the 36 patients receiving relaxin gel had spontaneous rupture of the fetal membranes. It has been hypothesised that relaxin may facilitate membrane rupture as collagenase and plasminogen activator secretion are stimulated by porcine relaxin in human amnion and chorion cells cultured *in vitro* (Koay et al 1983). However, this *in vitro* effect was described after an incubation period of 32 h and therefore 15 h may have been too short a time to study the effect of exogenous porcine relaxin on human fetal membranes *in vivo*.

Finally, this study provides further evidence that the human

cervix will recognise and respond to pure porcine relaxin despite there being only an approximate 50 percent homology in the amino acid structure between porcine and human relaxin (Hudson et al 1983). Paradoxically, human myometrial contractility is not inhibited by porcine relaxin (Chapter 10) but human cervical smooth muscle contractility is inhibited by porcine relaxin (Norstrom et al 1984). It is yet to be proven that human myometrium has relaxin receptors and that human relaxin will inhibit human myometrial activity. However, given that this is likely, the different response of the myometrium and the cervix in the human to porcine relaxin suggests that human relaxin receptors may be modulated in different ways in different target organs and thus allow the uterine body and the uterine cervix to function independently of each other and still be under the influence of the same hormone at different times during pregnancy.

CHAPTER 14

THE MORPHOLOGY OF CERVICAL RIPENING INDUCED BY THE HORMONES RELAXIN AND PROSTAGLANDIN $F_{2\alpha}$ IN A RABBIT MODEL

14.1 SUMMARY

In previous studies both purified porcine relaxin and prostaglandin $F_2\alpha$ have been applied vaginally in the human to promote cervical ripening near term. In this study the histological changes in the cervix induced by these locally applied hormones are described in a rabbit model. Similar histological changes occurred following treatment with relaxin or prostaglandin $F_2\alpha$ and these changes were comparable with those seen in the cervix following the spontaneous onset of labour in control rabbits. The main histological features were a dissolution of the collagen bundles and an apparent increase in the ground substance. However, a unique giant cell infiltrate was seen in the relaxin treated and spontaneously labouring control rabbits. The nature and possible function of these giant cells are discussed. The similarity of the general morphological changes in the cervix induced by relaxin and prostaglandin $F_2\alpha$ supports the concept that these hormones may act either in sequence or separately to activate the same collagenolytic system to produce the same effect in cervical connective tissue rather than acting in parallel to produce separate or complementary structural changes.

14.2 INTRODUCTION

In most mammals, prior to parturition, there are structural changes in the connective tissue of the cervix that lead to its altered distensibility, dilatation and softening that are collectively known as cervical ripening. In the human, several hormonal agents (Steiner & Creasy 1983) including vaginally applied purified porcine relaxin (Chapter 11) and prostaglandin $F_2\alpha$ (Appendix 1) have been given prior to the induction of labour to induce such changes in the cervix. These two hormones individually applied appear to produce a similar clinical effect and when given in combination there does not appear to be any additive effect (Chapter 12). This suggests that these hormones may act in sequence or separately, to produce the same effect rather than working in parallel to produce different or complementary changes in the cervix.

No study of the histological changes in the cervix following administration of pure porcine relaxin or prostaglandin $F_2\alpha$ has been published. This study examines and compares the histological changes induced by these hormones. For ethical reasons adequate material for the study could not be collected from the human. Therefore, the rabbit was chosen as it has been shown that this animal is a good model for the investigation of the mechanical behaviour of the cervix and the rabbit cervix appears to be comparable to the human cervix in its physiologic responses (Conrad & Hoover 1982; Danforth, Buckingham & Roddick 1960).

14.3 MATERIALS AND METHODS

Twelve mature female New Zealand White rabbits weighing 4.5-5.8 Kg were studied. Six of these served as untreated controls. Two of the control rabbits were not pregnant, two were sacrificed on day 28 of pregnancy, one was sacrificed in labour on day 29 of gestation and one 12 h postpartum on day 30 of gestation. Of the other six rabbits, two were treated with either 4 mg prostaglandin $F_2\alpha$ and four with 0.3 mg purified porcine relaxin. Both hormones were mixed in a tylose gel and administered vaginally by means of a syringe and catheter on day 27 of pregnancy. Fifteen hours later on day 28 of pregnancy the treated animals were sacrificed. The uterus was removed after sacrifice and the cervix and lower uterine segment fixed for histological examination. Paraffin sections of each cervix were cut in a sagittal plane and were stained with either haematoxylin and eosin, Masson's trichrome to highlight collagen or Alcian Blue - Periodic Acid Schiff (P.A.S.) which stains the mucopolysaccharides of the ground substance blue.

Tissues for electron microscopy were fixed in a 5% paraformaldehyde and 3% gluteraldehyde in cacodylate buffer, post fixed in 1.5% osmic acid, dehydrated in a series of ethanolic solutions, transferred to propylene oxide and then embedded in Epon. Sections were examined on grids with AE1801 electron microscope at initial magnifications of 1,700 to 40,000.

The doses of prostaglandin $F_2\alpha$ and porcine relaxin chosen were in proportion to body weight to the clinically effective dose of these hormones which produce cervical ripening when administered vaginally in the human (Chapter 11). The porcine relaxin used was purified as in Chapter 2.

14.4 RESULTS

14.4.1 Control Rabbits (Figure 27)

The non-pregnant rabbit cervix is a relatively small firm structure and the histology reflects this appearance in that the collagen, staining dark blue on Masson's stain, is densely packed around the small muscle bundles, which stain pink (Figure 27A). There is little ground substance between the collagen fibres and the collagen bundles run in relative unison around the cervical muscle.

In late pregnancy (day 28) the rabbit cervix is a larger softer structure than the non-pregnant cervix, being about twice its previous size. The collagen bundles are not so densely packed and this results in a lighter blue staining of these fibres (Figure 27B). The collagen bundles still run together in a uniform fashion around the muscle fibres and there is an apparent slight increase in the ground substance.

In labour (Figure 27C) the rabbit cervix is even softer and longer than in late gestation. There is marked submucosal oedema and separation of the collagen fibres deeper in the cervix. The collagen fibres seem to be in disarray with little uniformity in their direction. There is an apparent large increase in the ground substance and the blood vessels are more prominent. Giant cells which are described in detail below, appear for the first time. They are seen more commonly around the blood vessels and in the subepithelial layer, although they can be found throughout the depth of the cervix and extend into the lower uterine segment.

Twelve hours pospartum the rabbit cervix is still macroscopically large (approximately three times the non-pregnant size) but firmer in consistency than during labour. The muscle bundles

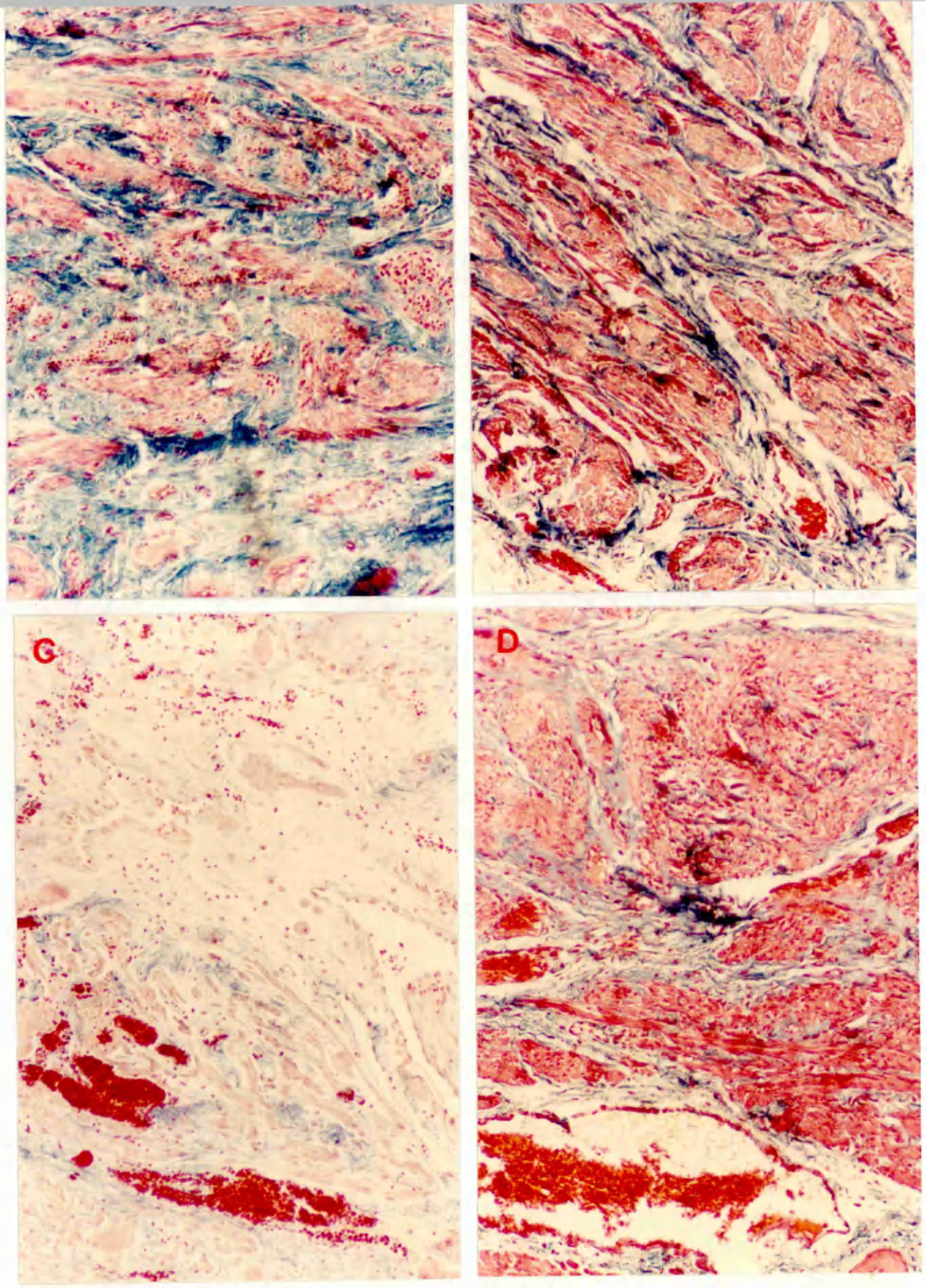


Figure 27 Cervical tissue from control rabbits.
Masson's trichrome stain (x80)
A. Non pregnant B. Day 29 pregnancy
C. During labour D. 12 hours postpartum

are more prominent and a large number of blood vessels are apparent (Figure 27D). The collagen fibres between the muscle bundles are more densely packed than during labour and are beginning to regain their uniformity of direction. There appears to be a corresponding reduction of ground substance and the giant cells seen in the cervix during labour could only occasionally be found.

14.4.2 Prostaglandin and Relaxin Treated Rabbits (Figure 28)

With the exception of the more frequent presence of giant cells in the relaxin treated cervices the histological changes induced by the two hormonal treatments are similar to each other and comparable to the changes seen in the cervix of the control rabbit in labour. All hormonally treated cervices responded in similar fashion. Macroscopically the cervices were large and soft. Histologically there is submucosal oedema and deeper in the cervical tissue there is an apparent marked increase in the ground substance, as demonstrated with Alcian Blue - P.A.S. stain. The collagen fibres do not run in uniform bundles but are generally widely separated without concurrence in their orientation. The blood vessles are more prominent in number and size suggesting an increase in vascularity. Only an occasional giant cell was seen in one of the prostaglandin treated cervices whilst numerous giant cells were seen in three of the four relaxin treated cervices. They are similar in morphology and general position to those seen in the cervices of labouring and post-partum control rabbits.

14.4.3 Giant Cells (Figures 29 and 30)

These cells do not conform to a precise description of any previously recorded cells and could not be positively classified

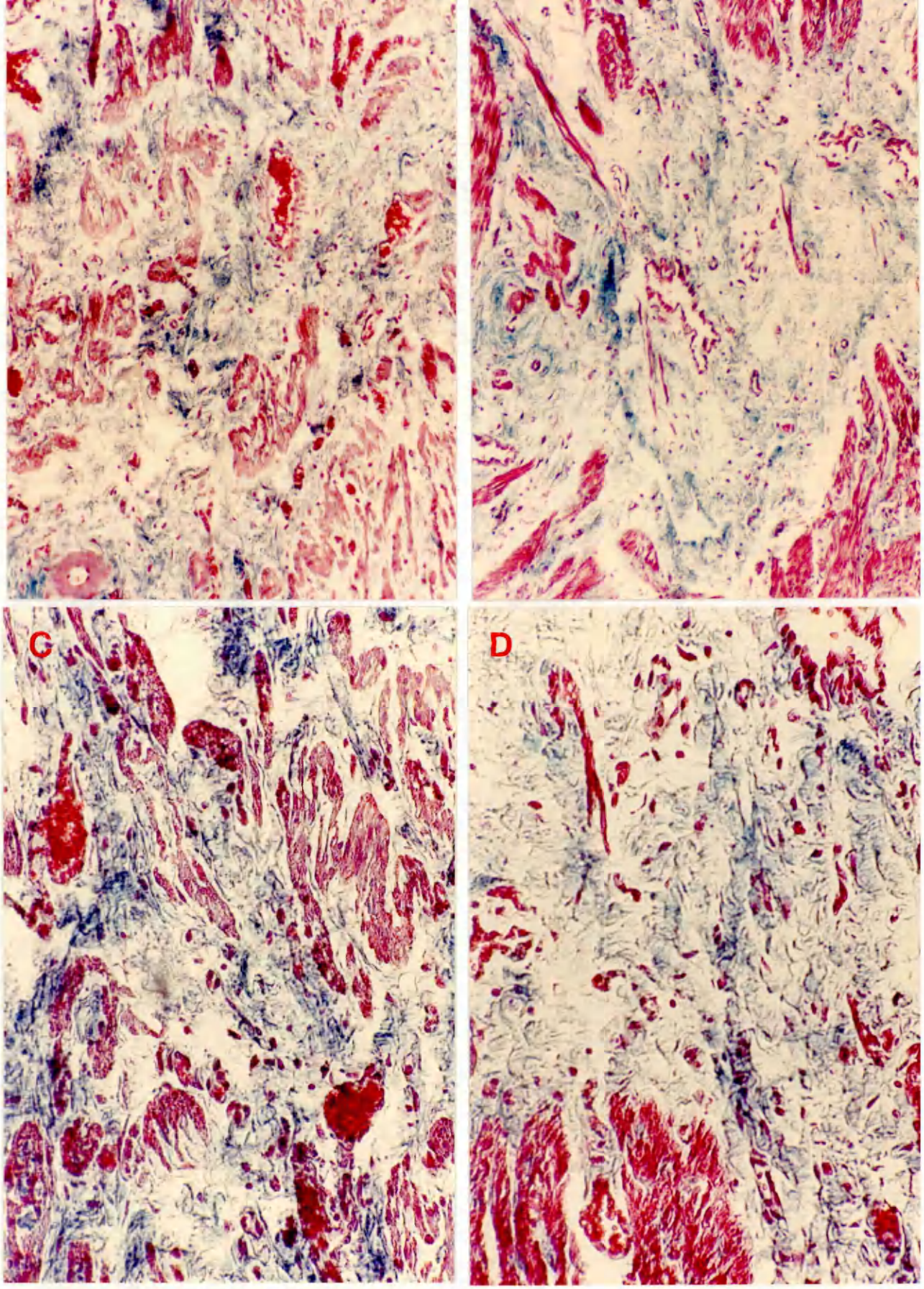
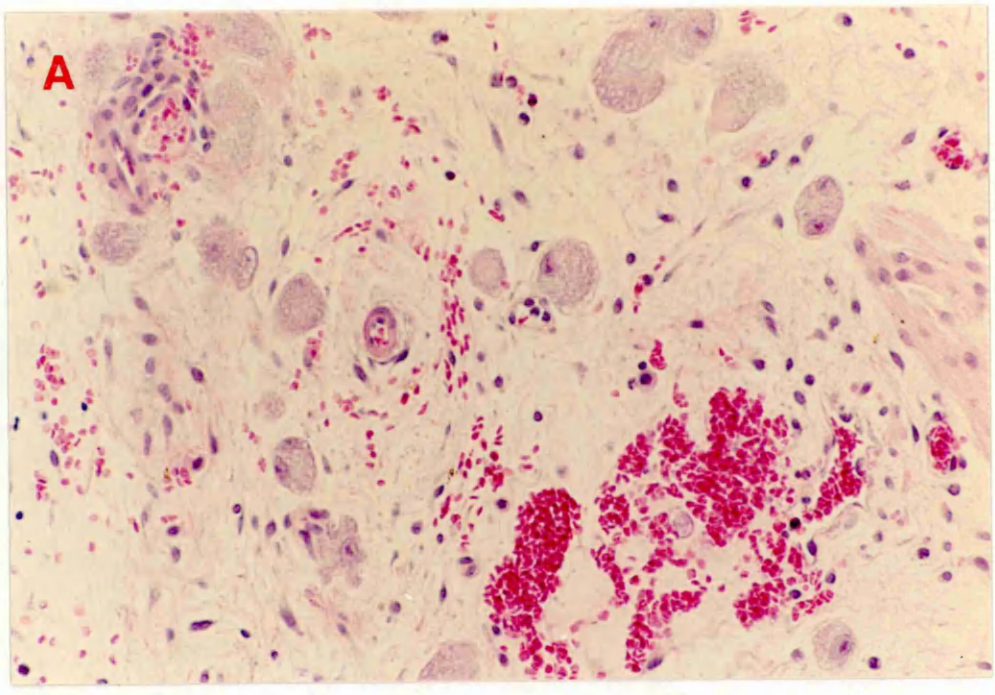


Figure 28 Cervical tissue from treated rabbits at day 28 of pregnancy.
Mason's trichrome stain
A. Prostaglandin $F_2\alpha$ treated (x80)
B. Relaxin treated (x80)
C. Prostaglandin $F_2\alpha$ treated (x200)
D. Relaxin treated (x200)

by any of four senior pathologists to whom they were shown. In this report we have simply called them giant cells because of their size (50-100 mμ in diameter) rather than definitively classifying them in any family of cells. There was some similarity in their appearance to ganglion cells and to decidual cells but neither neuronal nor uterine origin could be confirmed. They stain basophilic on haematoxolyin and eosin staining and are P.A.S. positive. They are multinucleated with large areas of vacuolation in the cytoplasm (Figure 29A, B). Ultrastructural examination showed these cells to be densely packed with ribosomes, mitochondria and rough-surfaced endoplasmic reticulum (Figures 30 and 31). Numerous small vesicles were seen throughout the cytoplasm but Golgi apparatus was not prominent and there were very few lysosomes. Multiple microvilli cover the surface of the cell and vesicles near the cell membrane suggest active pinocytosis. In some cells the nucleolus is relatively amorphous and in others the nucleolonema stands out clearly from the pars amorpha. Overall the ultrastructural picture is of a very active cell possibly involved in chemical or hormonal production and secretion.

These cells were seen frequently in the relaxin treated cervices and in the control cervix during labour. Only very occasional giant cells were seen in one of the prostaglandin treated cervices and in the postpartum control cervix. They were not seen in any of the non-pregnant or 28 day pregnant control rabbits. When present, these cells could be found throughout the connective tissue of the cervix but they appeared to be more prominent around blood vessels and in the submucosal layer. These cells were also found to extend into the lower uterine segments of the relaxin treated and labouring rabbits.



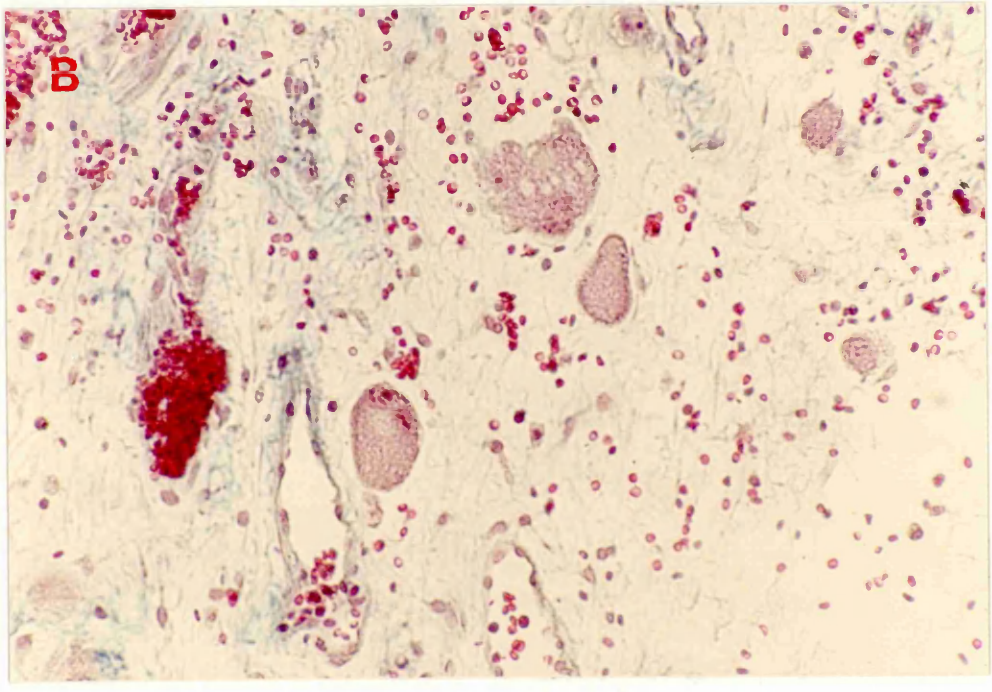


Figure 29 Giant cells in rabbit cervical tissue (x200).
A. Control rabbits in spontaneous labour (H. and E. stain).
B. Relaxin treated rabbit (Mason's trichrome stain).

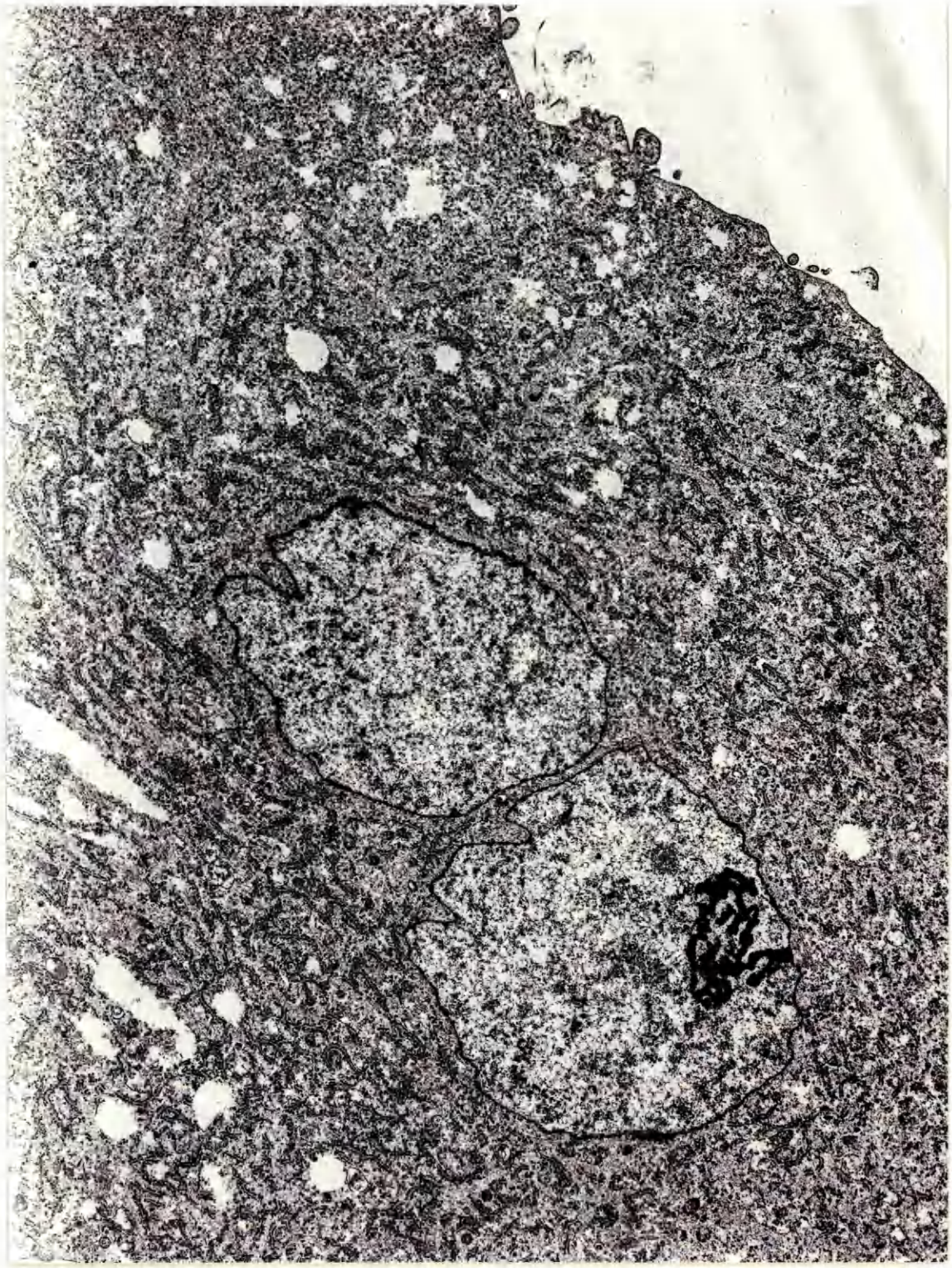


Figure 30 The ultrastructure of a giant cell in the cervical tissue of a pregnant rabbit treated with relaxin on day 28 of gestation (x7,500).

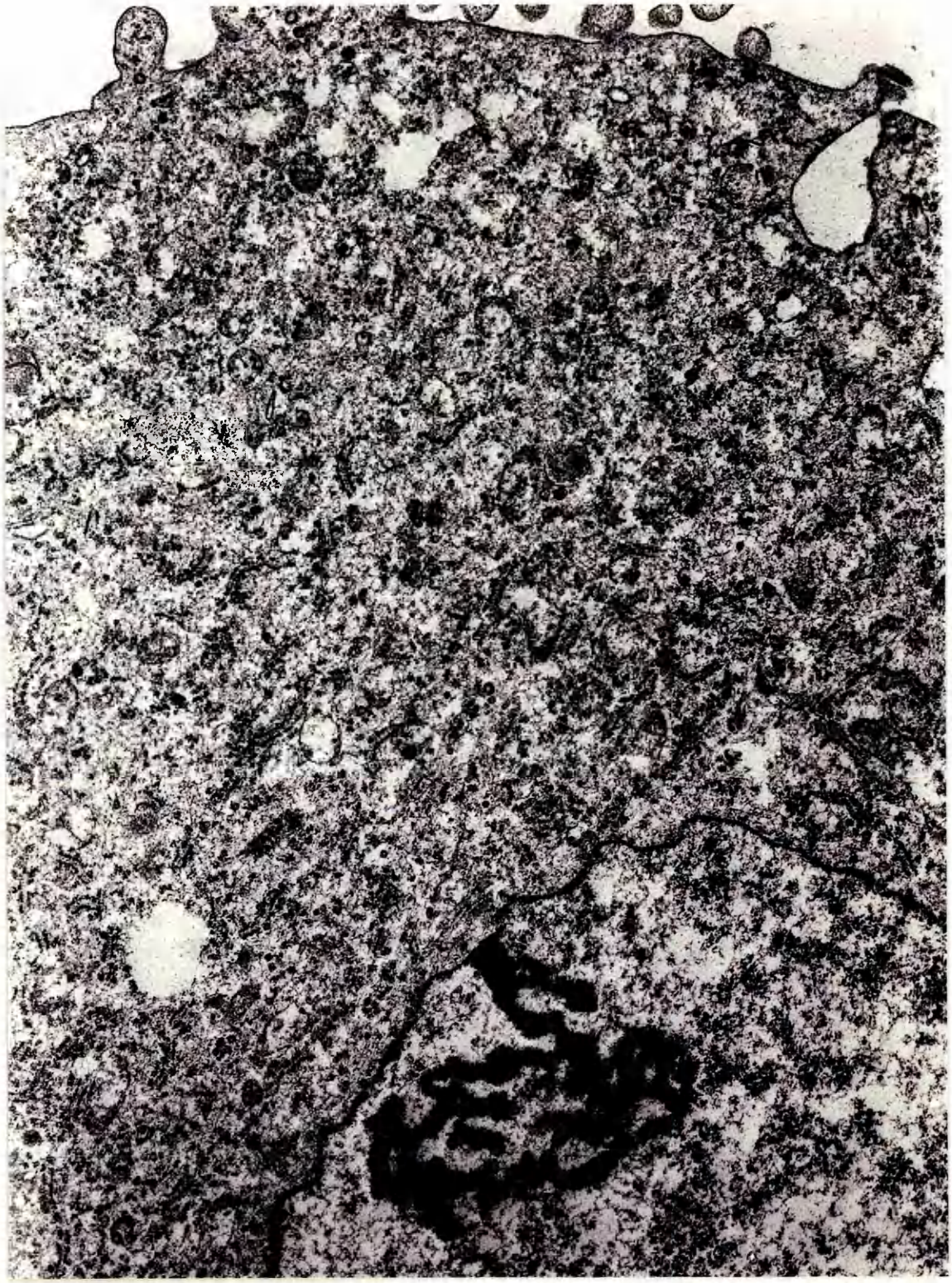


Figure 31 The ultrastructure of a giant cell in the cervical tissue of a pregnant rabbit treated with relaxin on day 28 of gestation (x18,900).

In these latter sections, the giant cells were found predominantly in the deeper layers of the decidua and between the muscle fibres of the inner myometrium.

14.5 DISCUSSION

This study shows that vaginally applied porcine relaxin and prostaglandin $F_2\alpha$ separately induce similar histological changes in the rabbit cervix. In clinical trials where porcine relaxin and prostaglandin $F_2\alpha$ were used in combination to induce cervical ripening, no additive clinical effect nor apparent synergism in their action was found (Chapter 12). This histological study supports the suggestion made in these clinical trials that relaxin and prostaglandin $F2^{\alpha}$ may act to produce the same structural changes in the term cervix rather than acting in parallel to produce separate or complementary cervical connective tissue changes. Whilst it is possible that relaxin and prostaglandins may act in sequence to produce this similar effect it is also possible that these hormones may separately stimulate the same collagenolytic process in cervical tissue near term. It has recently been shown that both prostaglandin (Norstrom & Wiqvist 1984) and relaxin (Norstrom et al 1983) increase cyclic adenosine 3^1, 5^1-monophosphate (cAMP) in human cervical tissue at the end of pregnancy and it has been suggested that the effects of these hormones on cervical connective tissue metabolism are mediated by cAMP. Thus, this histological study and previous clinical studies suggest that relaxin and prostaglandin $F_2\alpha$ may have similar morphological effects on the term cervix, even though the link or sequence of action between them is yet to be established.

The treatment-induced histological changes in the rabbit cervix were similar to those observed in the rabbit cervix during spontaneous labour, suggesting that relaxin and prostaglandin $F_2\alpha$ can induce the normal structural changes in the cervix associated with spontaneous cervical ripening and parturition. The hormonally induced changes described in this paper are comparable to the morphological changes in

needle biopsies of the human cervix in early pregnancy, following treatment with prostaglandin E_2. (Uldbjerg et al 1981). However, there have been no histological reports of the changes in the human cervix at term after treatment with prostaglandin $F_2\alpha$ or relaxin, presumably because of the difficulty in obtaining adequate cervical tissue at this time. As the rabbit has been shown to be a suitable model for studying cervical ripening (Conrad & Hoover 1982) it is suggested that the connective tissue changes induced by these hormones in the rabbit cervix, as described in this paper, are likely to reflect the morphological changes that occur in the human cervix after vaginal application of prostaglandin $F_2\alpha$ or relaxin.

The nature and function of the giant cell infiltrate is of some interest. These cells were mostly confined to the cervical and lower uterine segment sections from the rabbit in labour and three of the four rabbits treated with relaxin. Although these particular cells do not seem to have been described before in such a situation, different types of cells have been seen to invade the cervix during parturition in several species. Leukocytic infiltration has been described in the ovine cervix during parturition (Parry & Ellwood 1981). These cells are mostly neutrophils, but plasma cells, eosinophils and lymphocytes are also seen. In the guinea-pig and the human there is a leukocytic invasion of the cervix prior to parturition with a significant increase in the number of eosinophils (Liggins 1981). Janqueira et al (1980) hypothesise that the neutrophilic polymorphonuclear leukocytic invasion of the human cervix around the time of parturition contributes to the widespread collagenolysis occuring in the cervix at that time. They suggest that this action is mediated through the release of collagenase either from the leukocytes or indirectly from other cervical cells producing enzymes capable of digesting the extracellular matrix proteins. Other studies (Jones & Scott-Burden 1979) show

that macrophages are another potential source of such enzymes and that macrophage plasminogen activator promotes digestion of the extracellular matrix. Whether the giant cells described in the rabbit cervix near parturition are involved in collagenolysis cannot be determined from this study.

CHAPTER 15

THE ABSORPTION OF PORCINE RELAXIN FROM THE HUMAN VAGINA AND CERVICAL CANAL

15.1 SUMMARY

The systemic absorption of porcine relaxin administered in a tylose gel either vaginally or into the cervical canal to induce cervical ripening was measured by a homologous porcine relaxin radioimmunoassay. Peripheral serum samples were taken before and after treatment for up to 6 hours. A marked rise in serum relaxin levels was seen in 6 of the 12 patients treated with relaxin. The degree of cervical ripening associated with the relaxin treatment appeared to correlate with the absorption of the hormone. This is the first description of the absorption of a polypeptide hormone from the lower genital tract and suggests that relaxin's cervical ripening effect may be mediated either systemically or by direct action at the site of local application.

15.2 INTRODUCTION

The vagina is permeable to a wide variety of both organic and inorganic compounds and in the human has been used as a route of administration for steroid hormones and prostaglandins (Benziger & Edelson 1983). The vaginal absorption of a polypeptide hormone has not been documented in the human. The polypeptide hormone relaxin has recently been purified from porcine sources and has been tested in clinical trials as a cervical ripening agent when applied vaginally and intracervically. In doses of 2 mg mixed in a tylose gel it produces a significant degree of cervical ripening in approximately 85 percent of patients (Chapters 11 and 13). The aim of this study was to see if systemic absorption of porcine relaxin occurred from both the vagina and cervical canal and if so, to measure its rate of absorption as reflected by peripheral serum porcine relaxin levels when meausured in a homologous porcine relaxin radioimmunoassay.

15.3 PATIENTS AND METHODS

The absorption of porcine relaxin was measured in a total of 14 patients. Two mg of relaxin were instilled vaginally in a 5 ml tylose solution in 6 patients, 6 further patients were given the same dose of relaxin into the cervical canal in a 2 ml tylose solution and 2 patients received only a placebo (distilled water) in tylose gel (one by each route).

The systemic absorption of the porcine relaxin was measured by taking peripheral blood samples before treatment and hourly for up to 6 hours after application of the relaxin gel. Serum porcine relaxin was measured in the radioimmunoassay described in Chapter 4 which in this case was homologous for porcine relaxin. All samples in this study were run in the same assay.

The cervical score (modified Bishop's score (Calder et al 1977)) was assessed by the same observer both before treatment and 6 hours later.

15.4 RESULTS

No rise in baseline levels of serum relaxin occurred in either control patient receiving the placebo. Five of the 6 patients given porcine relaxin by the intracervical route showed a marked rise in their serum relaxin levels from 1 h after treatment and this rise persisted in some cases until the end of sampling (Figure 32). Improvement in the cervical score was noted in each patient when assessed again 6 h after treatment.

Only 1 of the 6 patients given relaxin by the vaginal route showed a definite rise in serum relaxin concentration following treatment (Figure 33). The cervical score of this patient improved 2 points in 6 h. Little or no improvement was seen over a 6 h period in the cervical scores of the remaining 5 patients. None of the 14 patients in this study commenced in labour during the 15 h following application of the gel.

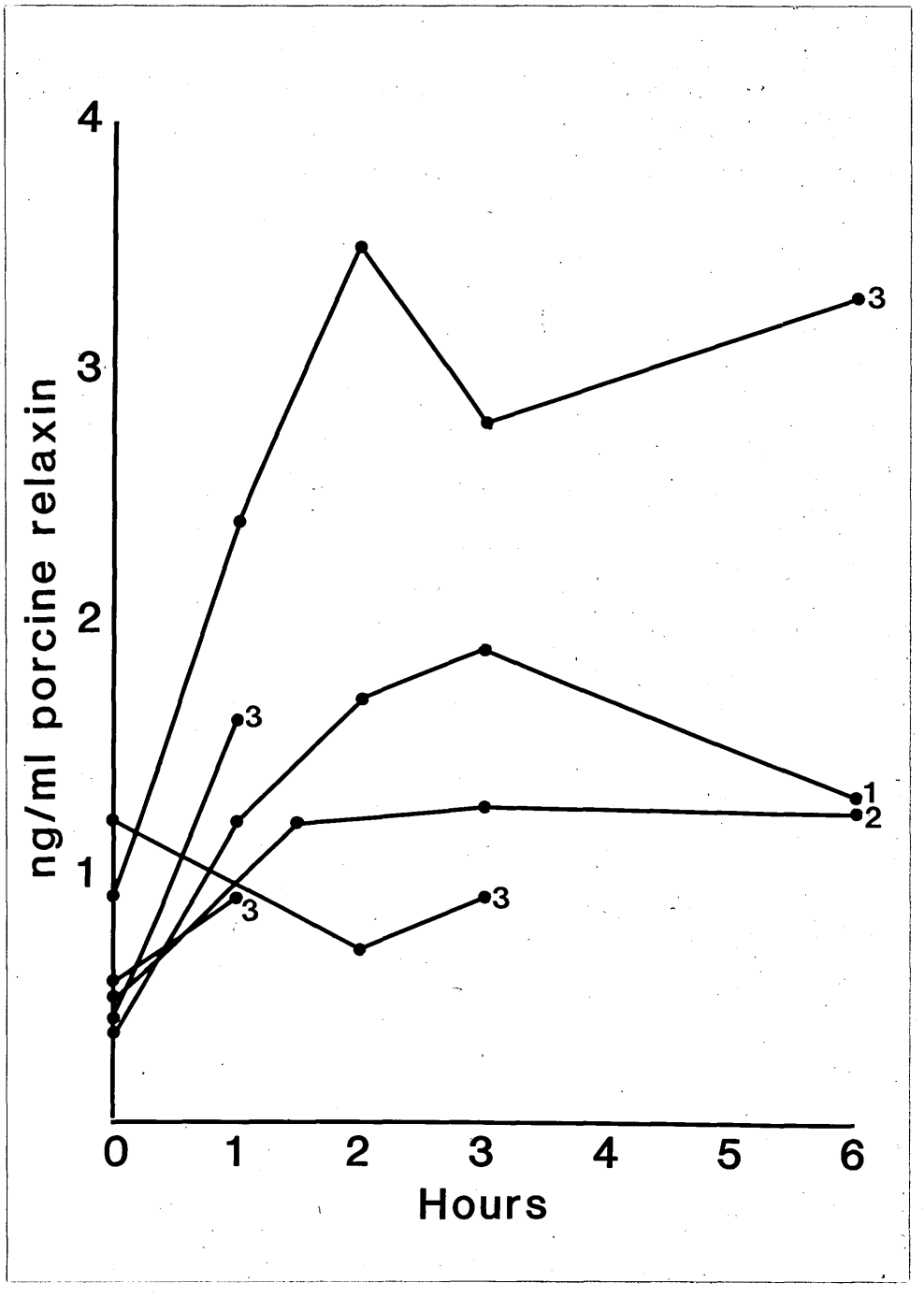


FIGURE 32 Serum relaxin concentrations as measured in a porcine radioimmunoassay following the intracervical application of 2 mg porcine relaxin. The figures in red indicate the improvement in the cervical score 6 hours after treatment.

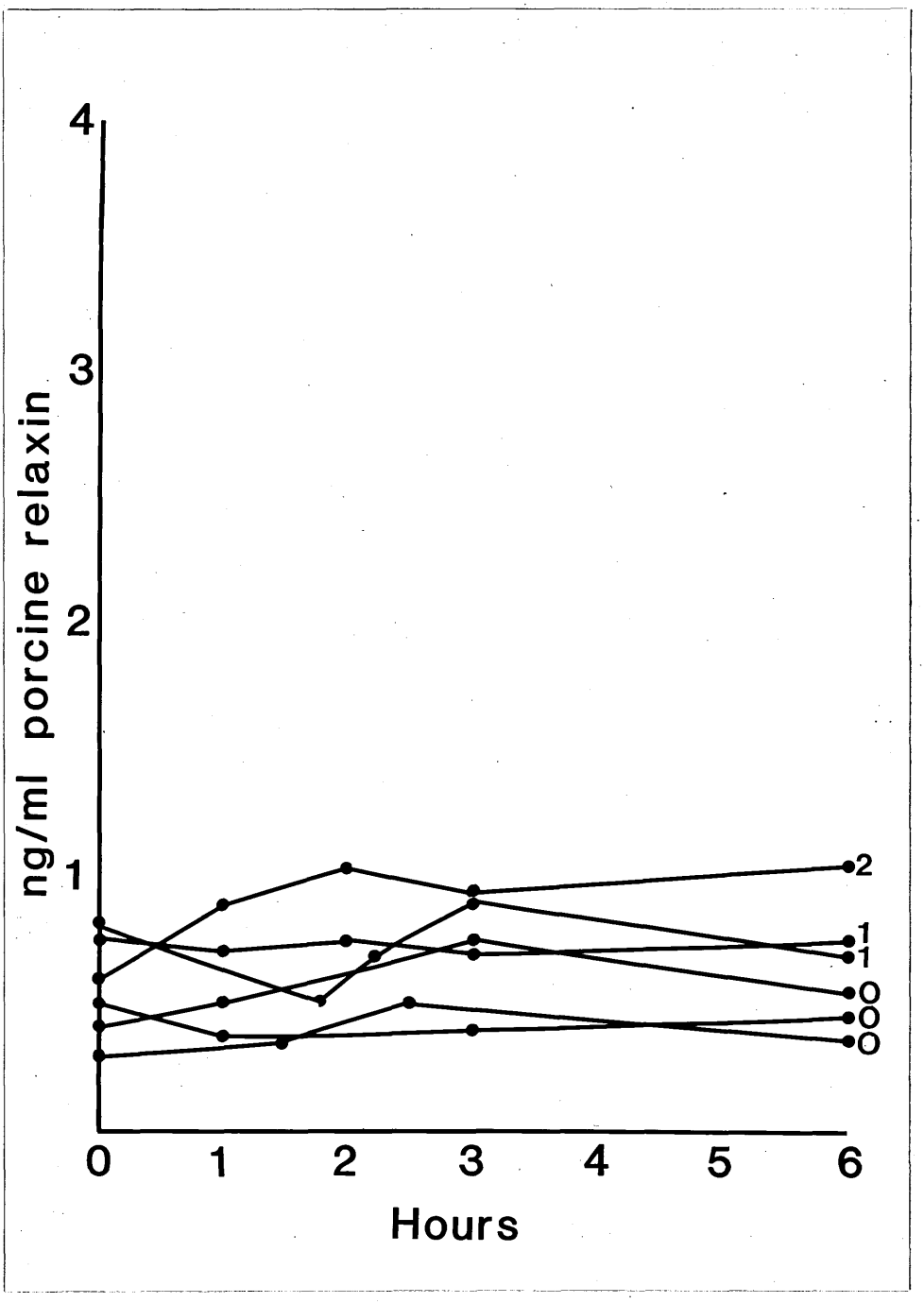


FIGURE 33 Serum relaxin concentrations as measured in a porcine radioimmunoassay following the vaginal application of 2 mg porcine relaxin. The figures in red indicate the improvement in the cervical score 6 hours after treatment.

15.5 DISCUSSION

This is the first study to show the absorption of a polypeptide hormone from the lower reproductive tract. It would appear from the small numbers in this study that absorption of porcine relaxin occurred more readily from the cervical canal than from the vagina. However, it should be noted that the cervical ripening effect was unusually poor in 5 patients given relaxin by the vaginal route. These patients showed an improvement of only 0-1 points in the cervical score whereas the mean improvement in larger studies using this route is 3.4 points (Chapter 11). Thus, these patients, may be atypical and further studies of peripheral serum levels are required (and are in progress) in patients who show a normal and significant degree of cervical ripening following the intravaginal instillation of porcine relaxin.

Nevertheless, a major rise in peripheral serum relaxin levels was seen in 6 of the 12 patients treated with porcine relaxin soon (1 h) after its application. The levels at 1 h were well above the normal range of relaxin in late pregnancy (Chapter 5) using the same assay and in this case, as the assay measures porcine relaxin, the increase is most likely to be due to the systemic absorption of the exogenous relaxin. No rise in endogenous relaxin was seen after application of the placebo gels.

This study suggests that the cervical ripening action of exogenously applied relaxin may be mediated either through its systemic circulation and/or its local absorption directly into the cervical tissues. However, clearly the absorption of relaxin, especially possibly the vaginal route, is variable and more research is required to ascertain the best route of administration and in particular the best carrier of the relaxin. Many new gels and pessaries for vaginal and intracervical prostaglandins are currently being tested through-

out the world and present studies in the author's laboratory are aimed at testing the *in vitro* and *in vivo* release rates of relaxin from water based gels and a hydrogel pessary.

CHAPTER 16

FOLLOW-UP STUDIES ON PATIENTS INVOLVED IN CLINICAL TRIALS OF PORCINE RELAXIN

16.1 SUMMARY

Whilst no short term side effects have been detected in the clinical trials reported in this thesis or elsewhere (Evans et al 1983), long term follow-up of patients treated with porcine relaxin is still underway. In particular, no involuntary infertility has yet been detected. Twenty-five patients have been sampled 3 to 12 months after treatment and no porcine relaxin antibodies have been detected in their serum. Screening for antibody formation is continuing.

16.2 INTRODUCTION

A concern of the candidate, the hospital's ethics committee and the Australian Department of Health was the possibility of any clinical side effects in the human trials. Permission for the clinical trials described in this thesis was only granted by the Australian Department of Health, the Australian National Biological Standards Laboratory, the Australian National Health and Medical Research Council and The Queen Victoria Hospital, Adelaide, Research and Ethics Committee, after considerable chemical, pharmacological and animal data had been presented and evaluated concerning the purification and clinical use of porcine relaxin. The strict quality control measures for the purification of porcine relaxin described in Chapter 2 have had to be maintained throughout the trials. (In 1982, the National Institutes of Health (U.S.A.) also gave permission for clinical trials with pure porcine relaxin and no side effects were reported in the first U.S. trial reported by Evans et al (1983)).

Patients in the cervical ripening and embryo transfer trials were carefully monitored, as previously described, during treatment, labour and their period of hospitalisation. No short term maternal or neonatal side effects were noted. However, a register of patients who have received porcine relaxin has been kept and long term follow-up is underway. Many patients who were in the first two cervical ripening trials have now conceived again and several have delivered a second child since the trial. No involuntary infertility has been reported or detected to date in patients who have received porcine relaxin.

The risks of side effects of any hormone are difficult to estimate when used in the human for the first time. However, the extensive use of very impure preparations of relaxin in the 1950's and 1960's when given parenterally had not been associated with apparent side

effects and a similarity could be drawn to the use of porcine insulin for many years until the recent introduction of human insulin. After repeated injections of porcine insulin antibodies to porcine insulin do occur in a small percentage of diabetics. As there is a greater similarity in the amino acid structure of porcine and human insulin than porcine and human relaxin it is considered important to check whether the single vaginal or intracervical administration of 2 mg porcine relaxin induces antibody formation in the human. To date 25 patients have volunteered to give serum samples for these checks 3 to 12 months after receiving porcine relaxin. Much larger numbers are currently being sampled.

16.3 METHODS

The radioimmunoassay described in Chapter 4 was modified as below. Serum samples were stored frozen at -80°C until used. 0.1 ml was incubated with 50 pg ^{125}I-labelled porcine relaxin at 4°C for 48 h. Anti-human gamma globulin (non gamma chain specific, precipitating antibody purchased from Antibodies Inc., California) was added, in two series in doses of 0.1 ml or 0.05 ml, to the serum with the labelled relaxin incubate. This was further incubated overnight at 4°C. These mixtures were then spun at 3000 rpm for 20 minutes on a Sorvall RC-2 centrifuge. The supernatant was decanted and a 0.4 ml cold barbitone buffer (pH 8.6, 0.05 M) was added to each tube and the precipitate resuspended. They were then recentrifuged as before and the supernatant decanted, the tubes wiped with cotton-tip and decanted. This avoided "trapping" of ^{125}I-relaxin in the precipitation matrix. Counts of the study and control samples in triplicate were performed as in Chapter 4.

16.4 RESULTS

None of the 25 study serum samples showed evidence of anti-porcine relaxin antibodies. The results are shown in Table 23.

PATIENT NUMBER	ANTIHUMAN GAMMAGLOBULIN	
	% bound ^{125}I-relaxin	
	0.1 ml	0.05 ml
1	5.2	1.4
2	4.8	3.9
3	6.9	4.1
4	5.6	2.1
5	2.5	2.6
6	6.2	2.3
7	4.6	2.0
8	4.4	1.6
9	7.1	1.9
10	9.5	2.7
11	7.3	1.8
12	9.7	2.2
13	7.5	0.77
14	9.4	0.61
15	8.3	2.9
16	9.0	1.1
17	4.6	1.2
18	10.1	1.5
19	2.3	2.2
20	4.3	2.3
21	5.3	1.9
22	3.8	3.7
23	7.8	2.2
24	3.8	2.2
25	3.7	1.5
Controls:		
A	8.3	4.0
B	10.9	4.9
C	4.5	3.1

TABLE 23 Results of percentage bound ^{125}I-relaxin in study and control patients being investigated for formation of porcine antibodies for two concentrations of precipitating antihuman gammaglobulin.

16.5 DISCUSSION

Although no patients have yet produced antibodies to porcine relaxin it is considered important to continue to check for this possibility. It is also unwise to assume that even if porcine relaxin antibodies are produced that they would not interfere with the role of human relaxin and thus the monitoring for long term side effects will be continued. Such surveillance is not simple and it is hard to comprehensively follow these patients when blood sampling and access for follow-up surveys is voluntary.

The use of porcine relaxin in clinical trials is relevant only to facilitate the study of human relaxin when it becomes available for clinical trials later this decade. The areas in which porcine relaxin has been successfully applied, the doses, carriers and routes used will all allow the quicker assessment of human relaxin's role in the human and therapeutic potential. Thus, when human relaxin is eventually available in sufficient amounts for clinical use it will perhaps be unnecessary to use pure porcine relaxin.

CHAPTER 17

FINAL DISCUSSION

17.1 GENERAL

Detailed discussion of each of the 12 studies presented in this thesis has been presented at the end of the relevant Chapter (Chapters 5-16). The candidate chose four main areas to study relaxin's possible role in human reproduction, namely peripheral serum relaxin levels during pregnancy, implantation of the blastocyst, myometrial contractility and cervical ripening prior to parturition. In a single thesis it has not been possible to comprehensively investigate all the possible roles of relaxin both before and during pregnancy. However, studies in the candidate's laboratories have also confirmed the data of Essig et al (1982) that porcine relaxin appears to enhance washed human sperm motility, both in vitro and in columns of human cervical mucus, and our preliminary data also suggests that pure porcine relaxin enhances the penetration of mouse ova by human sperm. Studies are in progress on the influence of relaxin on uterine stromal growth and uterine accommodation during pregnancy in the rat and on the possible role of relaxin in involution of the uterus during the puerperium. Studies in other laboratories (not the candidate's) involve investigation of relaxin's role in ovulation, fetal membrane rupture, breast development and lactation.

The possible roles of relaxin in the human are diverse but, so far, the areas being investigated are centred around its two main mechanisms of action which are (1) its probable influence on connective tissue remodelling in target tissues, and (2) its influence on cellular cyclic AMP, e.g. in the inhibition of myometrial contractility. The possible biological roles of relaxin have been summaries in Table 1, Chapter 1.

In this thesis peripheral serum relaxin-like immunoactivity, as measured in a porcine relaxin radioimmunoassay, has been shown for the

first time to decline steadily throughout the second half of pregnancy rather than remain stable as had been suggested in the few previous reports published. This decline is compatible with a waning role of relaxin in maintaining uterine quiescence. Even lower levels are reported in patients post term and in patients in premature labour. Whether such low levels contribute to the onset of parturition deserves much more study and is the subject of further project grant requests by the candidate in 1985. For the first time in the human statistically significantly higher levels of relaxin have been found in early labour compared to late pregnancy. Whilst this data needs to be confirmed by sequential studies, the suggestion is that the human is like all other animals so far investigated and has a peak of relaxin release and/or production near the onset of parturition. Such a peak may not contradict its postulated role in inhibiting myometrial contractility earlier in pregnancy if, as suggested in detail in the hypothesis below, the myometrium is responsive to oxytocin only at term and relaxin does not effectively inhibit oxytocin driven myometrial contractions. The rise in relaxin at parturition, however, allows the connective tissue changes in the cervix known as cervical ripening and this facilitates labour by decreasing the force needed by the oxytocin driven myometrial contractions to expel the fetus.

The radioimmunoassay data also suggests that the source of relaxin is maternal and that little or no relaxin crosses into the fetal circulation. Unusually high levels of maternal serum relaxin may be associated with excessive pelvic joint pain and laxity. This is the first report of an association between relaxin and such pelvic effects and warrants further investigation. In this study such high levels of relaxin in these patients were not associated with post-maturity supporting the hypothesis that myometrial contractility is not inhibited by high or low relaxin concentrations at term when the

myometrium comes under the influence of oxytocin.

The human studies in this thesis could not substantiate any role for relaxin in implantation. However, as discussed in Chapter 9, there were many possibilities why porcine relaxin by this route, dose and timing might have been inappropriate. Certainly in the rat exogenous relaxin influences implantation as described in Chapter 8 and relaxin levels are high in the human in early pregnancy. These early high levels in parallel and in conjunction with serum progesterone may on the other hand reflect its possible role in maintaining uterine quiescence by inhibiting myometrial contractility. However, this hypothesis awaits testing with human relaxin.

The most convincing data for a role for relaxin in the human was seen in the studies of cervical ripening and the initiation of parturition. Here both endogenous (Chapter 5) and exogenous (Chapter 15) relaxin levels rose in association with cervical ripening. In particular, exogenous porcine relaxin had a significant cervical ripening effect in most patients near term and initiated labour in up to one-third of these cases (Chapters 11 and 13). The similarity of the clinical outcome (Appendices 1, 2 and 3) and the similarity in the morphologically induced changes (Chapter 14) of prostaglandin $F_2\alpha$ and purified porcine relaxin suggests that there is a close link between these hormones in their mechanism of action and it seems likely that they may act either in sequence or separately to activate the same collagenolytic system to produce the same effect in cervical connective tissue. In an attempt to integrate what is known of relaxin's action in the human and in other animals with its postulated roles of (1) maintaining uterine quiescence to term, and (2) facilitating parturition, the following hypothesis is presented.

17.2 HYPOTHESIS

Relaxin's role in the prevention and initiation of labour (Figure 34)

At the time of writing (1984) the precise mechanisms involved in the initiation of human parturition remain an enigma. However, in animals, such as the sheep which have the placental enzyme 17α - hydroxylase, progesterone under the influence of fetal cortisol can be metabolised to 17α, 20α-dihydroxy progesterone. The increase in availability of 17α- hydroxylated C_{21} steroids in the placenta contributes to the sharp rise in unconjugated oestrogen concentrations in maternal blood prior to parturition. This increase in the oestrogen/progesterone ratio near term is thought to induce oestrogen receptors in the uterus and so increase cellular oestrogen in that organ. Although such a change in the serum oestrogen/progesterone ratio does not appear to occur in the human a change in the oestrogen/progesterone receptor status has not been excluded and a rise in cellular oestrogen in the uterine tissues near term is still possible.

However, in the human, Liggins (1984) postulates that an alteration in the balance between prostaglandin synthesis stimulators and inhibitors, probably initiated by the fetus, promotes an increase in the synthesis of prostaglandins from stores of arachidonic acid in the decidua and fetal membranes. Whether prostaglandins stimulate relaxin production or vice versa is not known but the work of Liggins (1984) and the data described in this thesis suggest they interact to stimulate the same collagenolytic mechanism in the cervix producing the connective tissue changes of cervical ripening.

Relaxin receptors appear to be induced in target tissues by

increasing levels of oestrogen. Thus, cervical stromal and muscle cells may become responsive to relaxin especially when the levels of both oestrogen and relaxin increase prior to parturition. Oestrogen also induces oxytocin receptors and in the human the myometrium becomes increasingly responsive to oxytocin towards term (Challis and Mitchell 1981).

The centre of this hypothesis (see Fig. 34) depends on whether human myometrium will respond to human relaxin as rat myometrium responds to rat or porcine relaxin, i.e. in the rat relaxin can inhibit spontaneous and prostaglandin driven myometrial contractility but not oxytocin driven myometrial contractility (Porter 1979a). Thus, at term, when either oestrogen or prostaglandins (depending on the animal, Fig. 34) initiate a rise in oxytocin receptors and the myometrium becomes responsive to oxytocin, relaxin cannot now inhibit uterine contractility and in conjunction with the cervical ripening effect of high levels of prostaglandin and relaxin, labour begins.

The candidate is aware, as partly discussed in Chapter 10, of other complex interactions of calcium, calmodulin and cyclic AMP together with the possible influence of myometrial cell gap junctions. However, the simplified hypothesis above is compatible with the known changes in these other factors (Challis & Mitchell 1981). Nevertheless, much has still to be learnt about the initiation of both premature and term labour in the human and I present this hypothesis to allow its testing in future projects (see below).

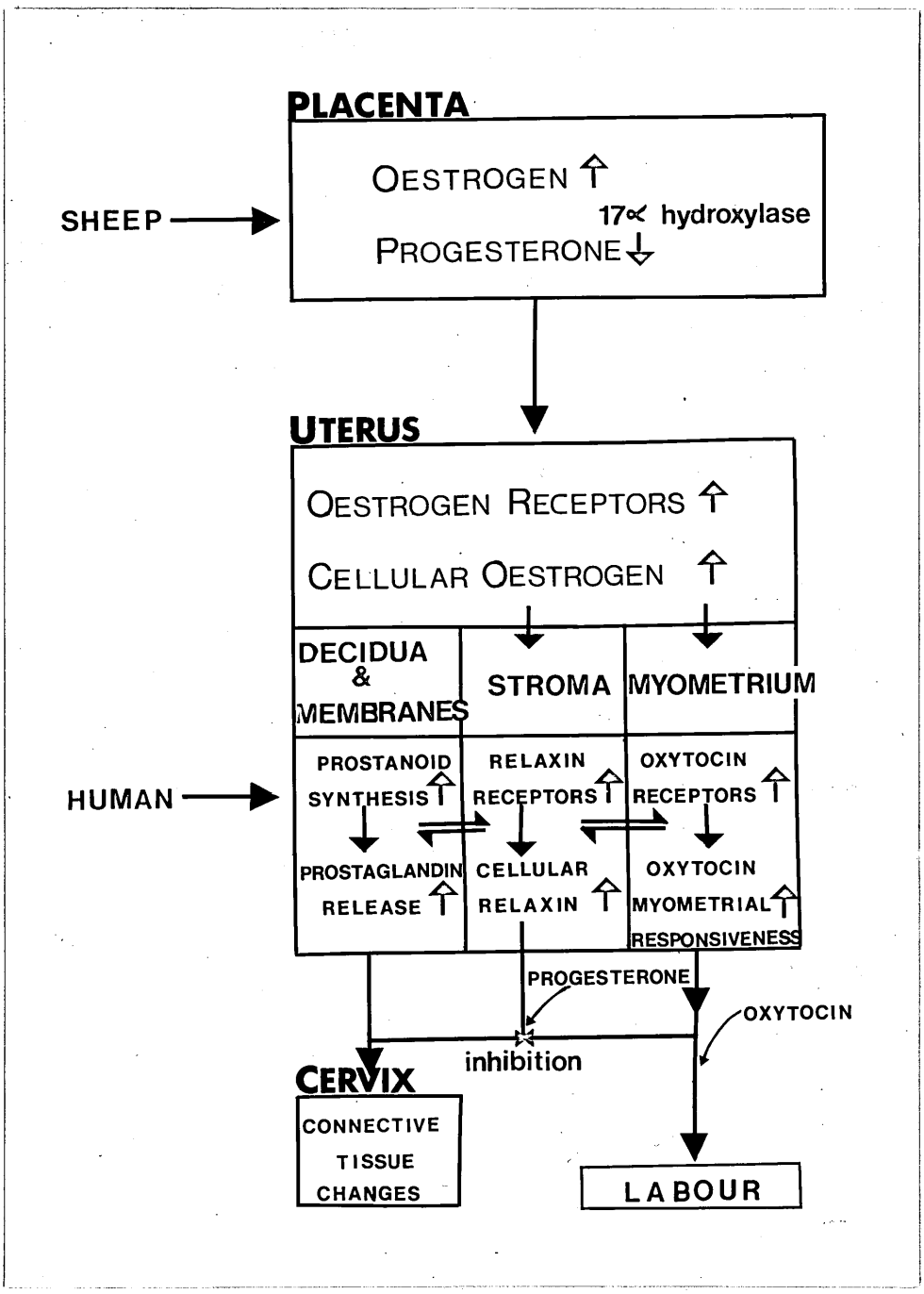


FIGURE 34 Relaxin's possible role in the prevention and initiation of labour. A hypothetical scheme of the relationship of relaxin to other hormones involved in parturition.

17.3 FUTURE RELAXIN RESEARCH

The production and availability of genetically engineered bioactive human relaxin for *in vitro*, animal and clinical trials will greatly help our understanding of the specific roles that relaxin plays in human reproduction. It is clear from many of the studies in this thesis that species specific relaxin will be necessary to confirm the roles suggested by the animal and *in vitro* studies using porcine relaxin. As detailed in Chapter 1 two relaxin genes have been identified in the human and the amino acid sequence of both of these has been deduced by the biochemists of the Florey Institute, Melbourne. Only one of these relaxins is thought to be biologically active (Dr. G. Tregear, personal communication). Small amounts of the potentially bioactive human relaxin have been synthesised and attempts are currently underway in the U.S.A. to synthesise large quantities of human relaxin using recombinant DNA technology.

With respect to the areas covered in this thesis human relaxin will be most valuable. Firstly if antibodies to human relaxin can be successfully raised a homologous human relaxin radioimmunoassay can be set up. Potentially this would be more sensitive than the porcine assay for human samples and hopefully specific for human relaxin allowing better correlation of relaxin levels in normal and abnormal pregnancy. Perhaps even more important than the estimation of peripheral serum levels will be the estimation of tissue levels of human relaxin, as relaxin may act as a local hormone. Access to human relaxin will also facilitate the development of a human relaxin receptor assay. More knowledge of relaxin receptors should greatly enhance our understanding of the mechanisms controlling relaxin.

The role of human relaxin in sperm motility, ovum penetration and implantation can also be helped by the use of species specific relaxin

and there may be merit in repeating the clinical trial described in Chapter 9 with human relaxin once safety and quality control studies on the genetically engineered relaxin have been conducted.

The hypothesis described earlier in this chapter concerning human relaxin's role in maintaining uterine quiescence until late pregnancy cannot be tested until human relaxin is available. Thereafter the *in vitro* experiments described in Chapter 10 would be valuable in quickly establishing whether human relaxin plays such a role. Lastly human relaxin should be used *in vitro* and *in vivo* studies to determine its effect on human cervical ripening and parturition. Experience gained in the porcine relaxin studies described in this thesis with regards routes of administration, carriers, doses, protocols, etc., will facilitate the studies with human relaxin. Once again safety studies and long term follow up of patients treated with human relaxin will be necessary.

The true role of human relaxin in human reproduction and any potential therapeutic application of it will have to be established by carefully conducted clinical trials. Clinical trials with human relaxin will have to be conducted slowly and critically. Small numbers of patients in such trials may give misleading results and generally only one of many clinical variables can be tested at one time. So it is important that commercial investors in this 'new' hormone do not demand quick results or make dubious claims before the hormone has been comprehensively investigated. For if relaxin is marketed as it was in the 1950's before its role was understood and before it had been fully investigated, then relaxin could undeservedly fall again into clinical disrepute. Nevertheless, relaxin appears to be involved in many important areas of human reproduction as described in this thesis and the long term therapeutic potential in understanding its potential roles in the human is very exciting.

APPENDIX 1

CERVICAL RIPENING AND INDUCTION OF LABOUR WITH INTRAVAGINAL PROSTAGLANDIN $F_{2\alpha}$

A1.1 SUMMARY

A randomised double blind trial was done to determine the effect on cervical ripening of 50 mg intravaginal prostaglandin $F_2\alpha$ ($PGF_2\alpha$) in a methyl cellulose gel given on the evening before surgical induction of labour. Patients were given either placebo or $PGF_2\alpha$ and in both groups cervical stretching and sweeping of the fetal membranes was attempted. Of the 40 control patients, 3 had gone into labour and the mean improvement in the cervical score was 1.6 before surgical induction the next morning. However, 20 of the 40 patients receiving $PGF_2\alpha$ went into labour before the proposed induction and the mean change in cervical score (5.1) was significantly greater than that in the placebo group. Of the 40 patients pretreated with $PGF_2\alpha$, 37 had improved cervical scores and significantly fewer required agumentation in labour with intravenous oxytocin than in the control group. No side effects were experienced and the patients found the treatment acceptable.

A1.2 INTRODUCTION

Surgical induction of labour in the presence of a long, closed, firm and posteriorly facing cervix when the presenting fetal part is high in the pelvis is usually associated with a high complication rate and a high intervention rate during labour, despite subsequent augmentation with intravenous oxytocin or prostaglandins. Fewer complications result when the cervix is favourable at the time of induction (Calder & Embrey 1973). Attempts to penetrate and locally pretreat the unfavourable cervix with laminaria tents (Cross & Pitkin 1978), oestradiol (Gordon & Calder 1977) and extraamniotic prostaglandin E_2 (Calder, Embrey & Tait 1977) before the induction of labour have met with some success. More impressive results were obtained when prostaglandin E_2 was given in a vaginal gel (McKenzie & Embrey 1977).

Induction of labour may lead to further intervention. Thus, efforts to decrease the trauma of induction and avoid subsequent intervention seems appropriate. The place of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) in the ripening of the cervix before induction of labour is not known. We have compared the effect of the application of exogenous $PGF_2\alpha$ with that of cervical stretching and stripping of the fetal membranes which itself causes a rapid rise in endogenous $PGF_2\alpha$ (Mitchell, Flint & Bibby 1977) and has often been claimed to be a method of improving cervical favourability or in some instances of precipitating labour.

A1.3 PATIENTS AND METHODS

A randomised double blind trial involving 80 patients was set up with the permission of The Queen Victoria Hopsital's research and ethics committee. Criteria for inclusion in the trial were: singleton pregnancy, cephalic presentation, an unscarred uterus, maternal height over 150 cm and informed consent from the patient. The patients were randomly allocated to two groups which received either 50 mg $PGF_{2\alpha}$ ('Prostin $F_{2\alpha}$') or a placebo (sterile water), the allocated constituent being mixed in 10 ml of a sterile solution of 8% methyl cellulose gel. In both groups stretching of the cervix and sweeping of the fetal membranes was attempted.

Random allocation was achieved by having a random list of two numbers. The numbers were sealed in envelopes by a third party and one of us (R.G.) confirmed the patient's eligibility for the trial before opening the envelopes in the randomised order to discover the treatment allocation. Close matching of the two groups was apparent by the end of the trial (Table A1).

A.H.M., unaware of the treatment allocation, assessed the cervical score (modified Bishop score (Calder et al 1977)) at 5 p.m. on the evening before proposed induction of labour. The viscous gel was then placed in the posterior vaginal fornix by means of a soft latex catheter and a syringe. The patient was asked to remain in bed for 1 h. Next morning at 8 a.m. the cervical score was reassessed by A.H.M. Thereafter, the labour was managed by obstetricians who were unaware of the constituents of the vaginal gel.

Statistical analysis of the results was carried out by means of the following tests, the Fisher exact test, the Mann-Whitney test and the χ_2 test.

VARIABLE	PLACEBO GROUP (n = 40)	PROSTAGLANDIN GROUP (n = 40)
Mean maternal age (yr)	27.0	27.2
Mean gestation (wk)	40.1	40.1
Mean parity	1.0	1.1
Mean birth weight (kg)	3.5	3.6
Mean cervical score at priming	5.2	4.7

TABLE A1 Patient matching in treatment and control groups

A1.4 RESULTS

Cervical stretch and sweep alone (i.e. the placebo gel group) resulted in the onset of labour in 3 out of 40 patients in that group (Table A2), whereas 20 of the 40 patients receiving prostaglandin $F_2\alpha$ with a cervical stretch and sweep started labour without further stimulation ($p<0.00002$, Fisher exact test). The average improvement in cervical score of the placebo gel group was 1.6, and in the prostaglandin group it was 5.1 ($p<0.00005$, Mann-Whitney test). The improvement in the cervical score was still significant even when the patients who had already delivered by 8 a.m. on the day of the proposed induction were excluded ($p<0.0001$, Mann-Whitney test). The cervical score remained unchanged in 20 of the control group whereas only 3 of the prostaglandin treated group showed no evidence of improvement ($p<0.00005$, Mann-Whitney test).

Significantly fewer women in the group pretreated with prostaglandin needed intravenous oxytocin to augment their labour ($p<0.01$ test). There were 26 normal deliveries in the group given the placebo. This result did not reach statistical significance. There was no significant difference in the average length of labours but there were more short labours in the prostaglandin treated group (Table A3). Three patients (all in the prostaglandin group) had labours lasting more than 12 h. The amount of analgesia required in labour, the incidence of fetal distress, the Apgar scores and the incidence of neonatal jaundice were not significantly different in the two groups. No side effects were noted in the group receiving vaginal $PGF_2\alpha$.

There was an improvement in the cervical score in the $PGF_2\alpha$ group whatever the gestation (Table A4), parity (Table A5), or initial cervical score (Table A6). An analysis of the patients who actually went into labour after the $PGF_2\alpha$ pretreatment showed that onset of labour was not

related to the patient's parity, gestation, or the favourability of the cervix at the time of priming. 5.1 (range 1-9) was the mean priming score of those who went into labour in the prostaglandin treated group without further stimulation and in those who did not go into labour after $PGF_2\alpha$ the mean priming score was 4.8 (range 1-9).

An analysis of all patients not in labour at the proposed time of surgical induction showed that those with a cervical score of 5 or less had a mean length of labour of 8 h and 40% had a normal delivery. Those with a cervical score of 6 or more had a mean length of labour of 6.4 h and spontaneous vaginal delivery in 63% of cases.

In both the treatment and the placebo gel groups some patients were aware of mild tightenings for up to 3 h after cervical stretching and application of the vaginal gel. Those experiencing contractions for more than 3 h after pretreatment generally proved to be in labour. At postnatal interview all the patients found this method of treatment acceptable.

OUTCOME	PLACEBO GROUP (n = 40)	PROSTAGLANDIN GROUP (n = 40)
Delivered at 15 h	3	13
Established in labour	0	7
Average change in cervical score	1.6	5.1
Average change in those undelivered	1.1	3.2
No. with no change	20	3
No. needing augmentation	26	10

TABLE A2 Outcome of cervical pretreatment on the morning of proposed surgical induction

OUTCOME	PLACEBO GROUP (n = 40)	PROSTAGLANDIN GROUP (n = 40)
Caesarean section	3	2
Instrumental delivery	16	12
Mean length labour* (hr)	6.5	6.75
Labour under 6 hr	12	19
Pethidine given (mean dose)	21 (116 mg)	25 (112 mg)
Epidural block (mean dose)	15 (20 ml)	12 (30 ml)
Apgar at 1 min.	7.9	7.8
Apgar at 5 min.	9.0	9.0

TABLE A3 Outcome of labour in control and prostaglandin treated groups

*Excluding Caesarean section

GESTATION (wk)	MEAN IMPROVEMENT IN CERVICAL SCORE	
	PLACEBO	PROSTAGLANDIN
35 - 38+	0.4 (5)	7.0 (4)
39 - 41	1.3 (26)	4.9 (28)
41+	1.7 (9)	4.9 (8)

TABLE A4 Gestation and mean change in cervical score 15 h after pre-treatment.

No. of patients in parentheses

PARITY	MEAN IMPROVEMENT IN CERVICAL SCORE	
	PLACEBO	PROSTAGLANDIN
0	1.1 (17)	5.2 (17)
1	1.4 (14)	5.9 (10)
2+	2.3 (9)	4.2 (13)

TABLE A5 Parity and mean change in cervical score 15 h after pre-treatment.

No. of patients in parentheses

INITIAL SCORE	MEAN IMPROVEMENT IN CERVICAL SCORE	
	PLACEBO	PROSTAGLANDIN
0 - 3	2.1 (11)	6.7 (12)
4 - 6	1.0 (18)	5.0 (18)
7 - 9	1.9 (11)	3.5 (10)

TABLE A6 Initial cervical score and mean change in cervical score 15 h after pretreatment.

No. of patients in parentheses

A1.5 DISCUSSION

Cervical stretching and sweeping of the membranes led to only a small improvement in the mean cervical score and only 3 out of 40 patients went into labour. Spontaneous labour might be expected in this proportion of women since most patients had reached their expected date of delivery. However, vaginal examination and stripping of the fetal membranes rapidly doubles the plasma concentrations of prostaglandin $F_{2\alpha}$ (Mitchell et al 1977). This manipulation may have been associated with the small improvement in the mean cervical score of the placebo group and may have potentiated the effect of the exogenous $PGF_{2\alpha}$ in the treatment group.

The trial was designed to compare the combined effect of exogenous and endogenous $PGF_2\alpha$ with the effect of stimulating a rise in endogenous prostaglandin concentrations alone. The addition of exogenous $PGF_2\alpha$ (50 mg) is apparently much more effective than cervical stretching and sweeping alone. The effect of vaginal prostaglandin gel without stripping of the fetal membranes is being investigated.

In 92% of the prostaglandin treated patients the cervical score improved and in 50% labour was initiated. These results are better than those reported when laminaria tents (Cross & Pitkin 1978), extraamniotic oestradiol (Gordon & Calder 1977) and extraamniotic prostaglandin E_2 (Calder et al 1977) were used for cervical ripening. However, a success rate similar to the one reported here was obtained with intravaginal prostaglandin E_2 (McKenzie & Embrey 1977).

The results of this study are in accord with the findings of several other groups (Calder & Embrey 1973; Turnbull & Anderson 1968) who demonstrated that patients with unfavourable cervical scores at surgical induction have longer labours with a lower spontaneous delivery rate than patients with more favourable cervical scores at

induction. However, in those patients given $PGF_2\alpha$ there was no correlation between cervical favourability, gestation or parity and the initiation of labour that evening. Thus, we cannot yet define a group in which vaginal $PGF_2\alpha$ without other intervention will result in labour.

There were more spontenaeous deliveries and more labours of short duration in the prostaglandin treated group than in the controls. Although these differences were not statistically significant, significantly fewer women in the PG group required intravenous oxytocin. Ambulation during labour is claimed to reduce the amount of analgesia required during labour, fetal distress and the operative delivery rate (Flynn, Kelly & Hollins 1978). During the present trial ambulation was not encouraged, to remove any effect that this might create. However, augmentation of labour with vaginal prostaglandin in a high-viscosity gel does not preclude ambulation in labour after an initial brief period of recumbancy to allow absorption. A combination of vaginal prostaglandin, radiotelemetry and ambulation allows induced, monitored but apparently "natural" labour to occur with a lower incidence of subsequent intervention than previously experienced when intravenous oxytocin was used to induce labour. Vaginal prostaglandins seem to make the induction of labour more simple, especially in the presence of an unfavourable cervix, but careful selection of patients is still necessary.

APPENDIX 2

A DOUBLE BLIND DOSE TRIAL OF INTRAVAGINAL PROSTAGLANDIN $F_{2\alpha}$ FOR CERVICAL RIPENING AND THE INDUCTION OF LABOUR

A2.1 SUMMARY

A randomised double blind trial involving 90 patients was set up to compare the efficacy of 25 mg $PGF_2\alpha$, 50 mg $PGF_2\alpha$ and a placebo on cervical ripening when given in a vaginal tylose gel on the evening before surgical induction of labour. Preliminary stretching of the cervix and sweeping of the fetal membranes was not undertaken. In the 30 control patients, labour was not initiated and the mean improvement in the cervical score before surgical induction the next morning was 0.86. In the group of 30 patients receiving 25 mg $PGF_2\alpha$, labour commenced during the night in 9 patients and the mean improvement in the cervical score was 3.76 ($p<0.0005$); the corresponding figures for the 30 patients receiving 50 mg of $PGF_2\alpha$ were 10 patients coming into labour and cervical score improvement of 4.63 ($p<0.0005$). The difference in the mean improvement of the cervical score between the two prostaglandin groups was not significant. Significantly fewer prostaglandin treated patients needed augmentation during labour with intravenous oxytocin ($p<0.025$) and there was a significant increase in the spontaneous delivery rate in the combined prostaglandin treated group ($p<0.025$). There was no statistical difference in the outcome of labour between the two prostaglandin groups. It was not possible to predict the patients whose cervices would not respond to $PGF_2\alpha$ pretreatment (15%) or those in whom labour would be initiated (30%). No side effects were experienced.

A2.2 INTRODUCTION

Intravaginal prostaglandin E_2 (PGE_2) and prostaglandin ($F_2\alpha$) have been used successfully to ripen the unfavourable cervix with a subsequent reduction in intrapartum complications (MacKenzie & Embrey 1977; MacLennan & Green 1979). Pretreatment with intravaginal prostaglandins is beneficial even in the presence of a favourable cervix (Mellows et al 1977; MacKenzie & Embrey 1978).

In Australia only $PGF_2\alpha$ is available and there is little documentation of the efficacy of different doses of this prostaglandin when given in vaginal gel before surgical induction of labour. In a previous trial (MacLennan & Green 1979) we reported that 50 mg intravaginal $PGF_2\alpha$ initiated labour in 50 percent of the patients studied and that 92 percent of the patients showed signs of cervical ripening following the pretreatment. In that trial, however, stretching of the cervix and sweeping of the fetal membranes was attempted in each patient and it was not determined whether this potentiated the effect of the exogenous prostaglandin.

Thus, a randomised dose trial has been set up to compare the effects of 50 mg $PGF_2\alpha$, 25 mg $PGF_2\alpha$ and a placebo gel given intravaginally, and to study their effectiveness in the absence of any additional manipulation of the cervix.

A2.3 PATIENTS AND METHODS

A randomised double blind trial involving 90 patients was set up with the permission of The Queen Victoria Hospital's research and ethics committee. Criteria for inclusion in the trial were singleton pregnancy, cephalic presentation, an unscarred uterus, maternal height over 150 cm, informed consent from the patient and an absence of any history of asthma (as theoretically, $PGF_{2\alpha}$ given vaginally may exacerbate this condition). The patients were randomly allocated to 3 groups receiving either 25 mg $PGF_{2\alpha}$ (Prostin $F_{2\alpha}$, Upjohn), 50 mg $PGF_{2\alpha}$ or a placebo (10 ml sterile water), the allocated constituent being made up to 10 ml with sterile water and mixed with 700 mg tylose granules (Hoechst) to make a viscous gel. Random allocation was achieved by having a random list of 3 numbers. The numbers were sealed in enveloped by a third party and one of us (R.G.) confirmed the patient's eligibility for the trial before opening the envelopes in the randomised order to discover the treatment allocation. Close matching of the 3 groups in all respects was apparent by the end of the trial (Table A7).

A.H.M., unaware of the treatment allocation, assessed the cervical score (modified Bishop score (Calder et al 1977)) at 5 p.m. on the evening before the proposed induction of labour. The viscous gel was then placed in the posterior vaginal fornix by means of a soft latex catheter and syringe. The patient was asked to remain in bed for 1 h. Next morning at 8 a.m. the cervical score was reassessed by A.H.M. Thereafter, the labour was managed by obstetricians unaware of the composition of the vaginal gel.

Statistical analysis of the results was carried out using Student's t test, the Fisher exact test and the χ_2 test.

VARIABLE	PLACEBO	25 mg $PGF_2\alpha$	50 mg $PGF_2\alpha$
Maternal age (yr)	26.1	26.0	26.2
Gestation (wk)	40.0	40.2	40.1
Parity	0.6	0.8	0.8
Birth weight (kg)	3.4	3.4	3.5
Initial cervical score	4.3	4.2	4.1

TABLE A7 Patient matching in control and pretreatment groups

Mean figures

A2.4 RESULTS

The effect of the 3 gels 15 h after vaginal application is shown in Table A8. Labour did not supervene in any of the patients pretreated with placebo, whereas 9 patients given 25 mg $PGF_2\alpha$ ($p<0.001$) and 10 patients given 50 mg $PGF_2\alpha$ ($p<0.0005$) laboured. The mean improvement in the cervical score was 0.86 for the placebo group compared with 3.76 for the 25 mg $PGF_2\alpha$ group ($p<0.0005$) and 4.63 for the group receiving 50 mg $PGF_2\alpha$ ($p<0.0005$). There was no statistical difference between the 2 prostaglandin groups as regards the initiation of labour or the improvement in the cervical score.

Significantly fewer patients needed augmentation with intravenous oxytocin after pretreatment with 50 mg $PGF_2\alpha$ ($p<0.0025$) and with 25 mg $PGF_2\alpha$ ($p<0.025$) than the placebo group (Table A9). There was an increased number of normal deliveries in both of the prostaglandin treated groups. The increased incidence of normal deliveries for the prostaglandin groups taken together was statistically significant ($p<0.025$). There was no statistical difference between any of the groups as regards the length of labour, analgesic requirements and Apgar scores.

A similar degree of cervical ripening was found for both prostaglandin doses when the results were grouped according to the initial cervical score (Table A10, parity (Table A11) and gestation (Table A12). The onset of labour was not related to cervical favourability, parity or gestation. On the other hand, there was no correlation of these factors with the patients who showed no signs of cervical ripening after prostaglandin pretreatment. However, it was our clinical impression that where the presenting part was high, cervical ripening following prostaglandin application was poor. There was a high incidence of surgical delivery and malposition of the fetal head in

labour among patients whose cervices did not respond to pretreatment.

No maternal or fetal side effects were recorded in either of the groups treated with $PGF_2\alpha$.. There was one neonatal death due to multiple major congenital anomalies in the 50 mg $PGF_2\alpha$ group. The indications for the caesarean sections in the prostaglandin groups were fetal distress (2) and cephalopelvic disproportion, and in the placebo group the indications were cervical dystocia, failure to progress and fetal distress.

OUTCOME	PLACEBO (n = 30)	25 mg $PGF_{2\alpha}$ (n = 30)	50 mg $PGF_{2\alpha}$ (n = 30)
Delivered within 15 h	0	6	5
Established in labour	0	3	5
Mean change in cervical score	0.86	3.76	4.63
Mean change where labour did not supervene	0.86	1.93	2.65
No. with no change	18	6	3

TABLE A8 Outcome of cervical pretreatment

OUTCOME	PLACEBO (n = 30)	25 mg $PGF_2\alpha$ (n = 30)	50 mg $PGF_2\alpha$ (n = 30)
No. augmented with oxytocin	23	15	12
Mean length of labour* (hr) (± S.D.)	8.2 (± 3.2)	7.8 (± 3.8)	7.3 (± 3.7)
Caesarean section	3	1	2
Instrumental delivery	15	9	10
Pethidine given (mean dose)	13 (115 mg)	17 (96 mg)	18 (97 mg)
Epidural block (mean dose)	15 (31 ml)	13 (20 ml)	8 (28 ml)
No analgesic in labour	4	6	8
Apgar at 1 min.	8.1	7.8	8.1
Apgar at 5 min.	9.0	9.1	9.1

TABLE A9 Outcome of labour in control and treatment groups

*Excluding Caesarean section

INITIAL SCORE	MEAN IMPROVEMENT IN CERVICAL SCORE		
	PLACEBO	25 mg $PGF_2\alpha$	50 mg $PGF_2\alpha$
0 - 3	0.9 (14)	3.7 (15)	5.6 (14)
4 - 6	0.9 (12)	4.5 (11)	3.8 (11)
7 - 9	0.5 (4)	2.6 (4)	3.4 (5)

TABLE A10 Initial and post-treatment cervical scores

No. of patients in parentheses

PARITY	MEAN IMPROVEMENT IN CERVICAL SCORE		
	PLACEBO	25 mg $PGF_2\alpha$	50 mg $PGF_2\alpha$
0	0.9 (16)	3.2 (13)	4.4 (15)
1	0.7 (11)	4.5 (12)	5.0 (9)
2+	1.3 (3)	3.4 (5)	4.9 (6)

TABLE A11 Parity and mean change in cervical score

No. of patients in parentheses

GESTATION (wk)	MEAN IMPROVEMENT IN CERVICAL SCORE		
	PLACEBO	25 mg $PGF_2\alpha$	50 mg $PGF_2\alpha$
38 - 39+	0.8 (13)	6.1 (5)	3.0 (9)
40 - 41	1.0 (12)	3.2 (17)	6.1 (14)
41+	0.6 (5)	3.1 (7)	4.1 (7)

TABLE A12 Gestation and mean change in cervical score

No. of patients in parentheses

A2.5 DISCUSSION

This trial confirms our preliminary report (MacLennan & Green 1979) of the efficacy of intravaginal $PGF_{2\alpha}$ as a cervical ripening agent. In the first trial, stretching and sweeping of the cervix, followed by 50 mg $PGF_{2\alpha}$ given intravaginally, initiated labour in half the patients and the mean improvement in the cervical score was 5.1. In comparison, in this trial where no cervical manipulation was attempted, one third of the prostaglandin pretreated patients laboured and the mean improvement in the cervical score was 4.6. Although the difference is not statistically significant ($0.5 < p < 0.1$) for the numbers involved in these trials, cervical manipulation may be responsible for the apparent small enhanced effect of the exogenous $PGF_{2\alpha}$ by further increasing endogenous prostaglandin. In the first trial, the placebo group also underwent cervical stretching and sweeping of the membranes and 3 of the 40 patients laboured with a mean cervical improvement of 1.6 compared to none of the 30 patients given placebo in the present trial and a mean cervical improvement of 0.9. Thus, such cervical manipulation at the time of pretreatment may potentiate to a small degree the effect of intravaginal $PGF_{2\alpha}$.

As regards the comparative effectiveness of the 25 mg and 50 mg $PGF_{2\alpha}$, there appears to be little difference in their effect on cervical ripening, the initiation of labour and reducing subsequent intervention in labour. The 25 mg dose appeared to be more effective than described by MacKenzie & Embrey (1979) in their trial of 16 patients, where 2 patients laboured after treatment and the mean improvement in the cervical score was 2.2.

Despite the general effectiveness of intravaginal $PGF_{2\alpha}$, there was still a small group (15 percent) in whom there was no apparent effect. This group had a higher incidence of labour complications and

required surgical intervention more often. It is possible that failure of the cervix to ripen after prostaglandin pretreatment may indicate the presence of a malposition or disproportion.

As noted in our first trial (MacLennan & Green 1979), it was not possible to predict those patients who would labour after vaginal application of the prostaglandin. A subsequent trial (MacLennan & Green 1980) suggests that where the cervix is favourable for surgical induction of labour, augmentation with vaginal prostaglandin after rupture of the membranes, rather than before, is more effective than conventional augmentation with intravenous oxytocin. If predictability of delivery and timing of labour is required, then in this favourable group, vaginal $PGF_2\alpha$ after surgical induction may be more appropriate than pretreatment the evening before surgical induction. However, where the cervix is not favourable and induction of labour is necessary, $PGF_2\alpha$ pretreatment appears to be beneficial in most instances.

APPENDIX 3

THE EFFECT OF INTRAVAGINAL PROSTAGLANDIN $F_{2\alpha}$ ON LABOUR AFTER SPONTANEOUS AND ARTIFICIAL RUPTURE OF THE MEMBRANES

A3.1 SUMMARY

The effect on labour of 50 mg intravaginal $PGF_2\alpha$ or a standard intravenous oxytocin regimen was compared in 2 randomised trials involving a total of 83 patients, 23 of whom had experienced spontaneous rupture of the membranes (S.R.O.M.) and 60 of whom had artificial rupture of the membranes (A.R.M.) to induce labour. In each trial, labour had not been initiated by membrane rupture alone. In both trials only 20 percent of the patients receiving $PGF_2\alpha$ required further augmentation of labour with intravenous oxytocin. The mean length of labour in patients receiving $PGF_2\alpha$ was 2.5 h shorter in the A.R.M. trial and 3.0 h shorter in the S.R.O.M. trial than the mean length of labour in patients receiving intravenous oxytocin ($p<0.01$). In the A.R.M. trial, the $PGF_2\alpha$ treated group had significantly less analgesic requirements ($p<0.001$). Although more normal deliveries occurred in the patients treated with $PGF_2\alpha$ than oxytocin in both trials, the numbers did not reach statistical significance.

No side effects occurred in the $PGF_2\alpha$ treated patients or their babies and this method was much preferred by patients and nursing staff alike.

A3.2 INTRODUCTION

Intravaginal prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) have been used successfully as cervical ripening agents (MacKenzie & Embrey 1977; MacLennan & Green 1979). However, there are no published trials on the use of intravaginal prostaglandins to induce labour following either spontaneous rupture of the membranes (S.R.O.M.) or artificial rupture of the membranes (A.R.M.). Randomised trials were threfore designed to compare the efficiency of 50 mg intravaginal $PGF_{2\alpha}$ with intravenous oxytocin in patients where rupture of the membranes alone had not initiated labour. Patients whose membranes rupture spontaneously before the onset of labour are more prone to intrapartum complications, e.g. fetal malposition, prolonged labour and infection. Thus, as the outcome of labour in these patients might be different from those having a surgical induction of labour, separate randomisation of the treatments has been carried out for spontaneous and artificial rupture of the membranes.

A3.3 PATIENTS AND METHODS

Eighty-three patients not in labour, 23 after S.R.O.M. and 60 following A.R.M. were randomly allocated to groups receiving either intravenous oxytocin or 50 mg intravaginal $PGF_{2\alpha}$ mixed with 700 mg sterile tylose granules (Hoechst) to make a viscous gel. Random allocation was achieved by having 2 random lists of 2 numbers. A separate list was used for each mode of onset of membrane rupture. The numbers were sealed in envelopes by a third party and the patient's eligibility for the trial was confirmed before the envelopes were opened in the randomised order to discover the treatment allocation. The cervical score (modified Bishop score (Calder et al 1977)) was assessed by the same observor (R.G.) in each patient. Close matching in the A.R.M. group occurred by the end of the trial and to a lesser extent in the S.R.O.M. group (Table A13).

Criteria for inclusion in the trial were singleton pregnancy, cephalic presentation, an unscarred uterus, maternal height over 150 cm, no history of asthma, informed consent from the patient, and an absence of signs of labour. The timing of and necessity for stimulation of labour was left to the discretion of the obstetrician in charge who made this decision before the random selection of the method was made.

The same intravenous oxytocin regimen was used in each patient allocated this treatment, the infusion commencing at 3.33 mμ/minute and increasing every 15 minutes until labour was established or to a maximum of 40.0 mμ/minute. Patients were given intravaginal $PGF_{2\alpha}$ by means of a soft latex catheter placed in the posterior vaginal fornix. No attempt was made in this trial to stretch the cervix and strip the fetal membranes. The patients receiving $PGF_{2\alpha}$ were asked to remain in bed for 1 h, but thereafter were allowed to ambulate if they wished,

fetal heart monitoring being carried out by radiotelemetry. The mean length of ambulation in the prostaglandin group was 1 h. If labour was not satisfactorily established 4 h after application of the $PGF_{2\alpha}$ intravenous oxytocin was commenced. Statistical analysis of the results was carried out using Student's t test, the Fisher exact test and the χ_2 test.

CLINICAL FEATURES	A.R.M.*		S.R.O.M.*	
	$PGF_2\alpha$ (n = 10)	OXYTOCIN (n = 10)	$PGF_2\alpha$ (n = 10)	OXYTOCIN (n = 13)
Mean gestation (wk)	40.5	40.5	39.6	38.9
Parity	0.7	0.7	1.5	0.5
Mean cervical score at treatment	5.9	5.9	5.5	4.2
Mean membrane rupture/treatment interval (hr)	1.8	2.0	10.4	15.8
Birth weight (kg)	3.6	3.5	3.0	3.4

TABLE A13 Matching of treatment groups

A3.4 RESULTS

The outcome of labour after intravaginal $PGF_2\alpha$ and intravenous oxytocin following A.R.M. is shown in Table A14 and that following S.R.O.M. is shown in Table A15. In the A.R.M. group there is a statistically significant improvement in the outcome of the $PGF_2\alpha$-treated patients with respect to the length of labour and their analgesic requirements during labour. In the S.R.O.M. group only the length of labour was significantly reduced. When the A.R.M. and the S.R.O.M. groups are added together, the reduction in the mean length of labour of the $PGF_2\alpha$-treated patients becomes highly significant ($p<0.01$) compared with the oxytocin group, but the increase in spontaneous vaginal deliveries amongst the $PGF_2\alpha$-treated patients did not quite reach statistical significance ($0.05 < p < 0.1$, χ_2 test). Nine babies in the oxytocin groups required phototherapy for the treatment of neonatal jaundice, whilst only 3 babies in the $PGF_2\alpha$-treated group required this treatment; however, this difference was not significant.

No side effects were recorded in either treatment group and in particular there was no difference in the incidence of puerperal pyrexia. There was a marked patient preference for vaginal gel rather than the intravenous drip. No caesarean sections were performed on patients given $PGF_2\alpha$, whereas this procedure was necessary in 4 patients who were treated with intravenous oxytocin. The reasons given for the sections were: failure to progress (2), cephalopelvic disproportion (1), and fetal distress (1).

OUTCOME	$PGF_{2\alpha}$ (n = 30)	OXYTOCIN (n = 30)	P VALUE	STATISTICAL TEST
No. needing I.V. oxytocin	6	30	<0.0001	χ^2
Mean treatment/delivery interval (hr)	6.9	9.0	N.S.	---
Mean length of labour (hr)	6.0	8.5	<0.05	t
No. requiring no analgesia	16	4	<0.001	Fisher exact
No. requiring pethidine	13	22	<0.0025	χ^2
No. requiring epidural	3	12	<0.01	Fisher exact
Spontaneous vaginal delivery	22	17	N.S.	---
Instrumental delivery	8	12	N.S.	---
Caesarean section	0	1	N.S.	---
Apgar at 1 min.	8.1	8.1	N.S.	---
Apgar at 5 min.	9.0	9.0	N.S.	---
Babies requiring phototherapy	2	7	N.S.	---

TABLE A14 Outcome of labour after intravaginal $PGF_{2\alpha}$ and intravenous oxytocin following A.R.M.

OUTCOME	$PGF_2\alpha$ (n = 10)	OXYTOCIN (n = 13)	P VALUE	STATISTICAL TEST
No. needing I.V. oxytocin	2	13	<0.0001	Fisher exact
Mean treatment/delivery interval (h)	7.8	9.5	N.S.	---
Mean length of labour (h)	5.9	8.9	<0.05	t
No. requiring no analgesia	3	4	N.S.	---
No. requiring pethidine	7	9	N.S.	---
No. requiring epidural	1	3	N.S.	---
Spontaneous vaginal delivery	8	8	N.S.	---
Instrumental delivery	2	2	N.S.	---
Caesarean section	0	3	N.S.	---
Apgar at 1 min.	7.4	7.0	N.S.	---
Apgar at 5 min.	8.9	9.0	N.S.	---
Babies requiring phototherapy	1	2	N.S.	---

TABLE A15 Outcome of labour after intravaginal $PGF_2\alpha$ and intravenous oxytocin following S.R.O.M.

A3.5 DISCUSSION

The results of this study suggest that intravaginal $PGF_2\alpha$ is an effective method of stimulation of labour. Only 8 of the 40 prostaglandin treated patients were deemed by the obstetrician to need further augmentation with intravenous oxytocin. Few of these patients required intravenous therapy for ketosis or dehydration and thus the inconvenience and discomfort of intravenous therapy during labour was generally avoided. The vaginal gel was clearly the more popular method amongst the patients and the nursing staff involved in the trial.

In both the A.R.M. and the S.R.O.M. groups, labour was significantly shorter in those given $PGF_2\alpha$ and yet this did not seem to be at the expense of a stronger and more painful labour, since in the A.R.M. trial, the analgesic requirements were significantly reduced. Although the prostaglandin-treated patients were free to ambulate during their labour, this aspect of their management was not mandatory and the average length of ambulation was only 1 h. However Flynn & Kelly (1978) have shown that labour is shortened in the ambulant patient, there is less analgesic requirement and there are more normal deliveries. Thus, ambulation may have been a factor in the improved outcome of the prostaglandin-treated groups.

The tendency in the prostaglandin-treated groups towards an increase in spontaneous vaginal deliveries compared with the control group is similar to the findings in 2 other randomised trials we have reported, where intravaginal prostaglandins were given prior to A.R.M. (Appendices 1 and 2). All our trials were randomised in the same fashion and when the figures in each trial are totalled, 94 of 140 patients (67%) given vaginal $PGF2\alpha$ had a normal vaginal delivery compared with 58 of 113 patients (51%) given intravenous oxytocin after membrane rupture ($p<0.025$).

The apparent extra cost of $PGF_2\alpha$ in direct comparison to oxytocin is more than offset when an account is made of the associated costs in intravenous oxytocin, e.g. I.V. fluids, giving sets, intravenous cannulae, drip counters, pumps and nursing time. Further large cost benefits can be claimed for $PGF_2\alpha$ therapy from the point of view of shorter labours, decreased analgesic requirements, and the apparent increase in the normal delivery rate. Larger trials will be necessary to show whether neonatal jaundice is significantly reduced after induction of labour with $PGF_2\alpha$ compared with intravenous oxytocin.

REFERENCES

Abramowitz, A., Money, W., Zarrow, M.X., Talmage, R., Kleinholz, L. & Hisaw, F.L. (1944) Preparation, biological assay and properties of relaxin. Endocrinology, 34, 103-114.

Abramson, D. & Reid, D.E. (1955) Use of relaxin in treatment of threatened premature labour. Journal of Clinical Endocrinology, 15, 206-209.

Atele, S., Bryant-Greenwood, G.D., Chamley, W.A. & Day, E.M. (1979) Plasma relaxin immunoactivity in the pig at parturition and during nuzzling and suckling. Journal of Reproduction and Fertility, 56, 451-457.

Beck, Q., Adler, P., Szlachter, N., Goldsmith, L.T., Steinetz, B.G. & Weiss, G. (1982) Synergistic effect of human relaxin and progesterone on human myometrial contractions. International Journal of Gynaecology and Obstetrics, 20, 141-144.

Bedarkar, S., Turnell, W.G., Blundell, T.L. & Schwabe, C. (1977) Relaxin has conformational homology with insulin. Nature, 270, 449-451.

Beier, H.M. (1982) Uteroglobin and other endometrial proteins: biochemistry and biological significance in beginning pregnancy. In Proteins and Steroids in Early Pregnancy, eds. Beier, H.M. & Karlson, P. pp 39-72. Heidelberg: Springer Verlag.

Benziger, D.P. & Edelson, J. (1983) Absorption from the vagina. Drug Metabolism Review, 14, 137-168.

Bigazzi, M. (1981) Human decidual relaxin: The decidua as an endocrine gland. In Relaxin, eds. Greenwood, F.C., Bryant-Greenwood, G.D. & Niall, H.D. pp. 187-197. Amsterdam: Elsevier.

Bigazzi, M., Nardi, E., Petrucci, F. & Scarselli, G. (1983) Synthesis of relaxin by human decidua. In Biology of Relaxin and its Role in the Human, eds. Bigazzi, M., Greenwood, F.C. & Gasparri, F. pp. 206-212. Amsterdam: Excerpta Medica.

Bryant, G.D. (1972) The detection of relaxin in porcine, ovine and human plasma by radioimmunoassay. Endocrinology, 91, 113-1117.

Bryant-Greenwood, G.D. (1981) Relaxin from non-corpus luteum sources. In Relaxin, eds. Greenwood, F.C., Bryant-Greenwood, G.D. & Niall, H.D. pp. 171-177. Amsterdam: Elsevier.

Bryant-Greenwood, G.D. (1982) Relaxin as a new hormone. Endocrine Reviews, 3, 62-90.

Bryant-Greenwood, G.D. & Greenwood, F.C. (1979) Specificity of radioimmunoassays for relaxin. Journal of Endocrinology, 81, 239-247.

Calder, A. & Embrey, M.P. (1973) Prostaglandins and the unfavourable cervix. Lancet, ii, 1322-1323.

Calder, A.A., Embrey, M.P. & Tait, T. (1977) Ripening of the cervix with extraamniotic prostaglandin E_2 in viscous gel before induction of labour. British Journal of Obstetrics and Gynaecology, 84, 264-268.

Calguneri, M., Bird, H.A. & Wright, V. (1982) Changes in joint laxity

occuring during pregnancy. Annals of Rheumatic Disease, 41, 126-128.

Casten, C.G. & Boucek, R.J. (1958) Use of relaxin in the treatment of scleroderma. Journal of the American Medical Association, 166, 319-324.

Challis, R.G. & Mitchell, B.F. (1981) Hormonal control of preterm and term parturition. Seminars in Perinatology, 5, 192-202.

Cheah, S.H. & Sherwood, O.D. (1980) Target tissues for relaxin in the rat: tissue distribution of injected ^{125}I-labelled relaxin and tissue changes in cAMP levels after in vitro relaxin incubation. Endocrinology, 106, 1203-1209.

Chihal, H.J. & Epsey, L.L. (1973) Utilization of the relaxed symphysis pubis of guinea pigs for clues to the meachnism of ovulation. Endocrinology, 93, 1441-1445.

Cohen, N. (1963) Relaxin: studies dealing with isolation, purification and characterisation. Transactions of the New York Academy of Sciences, 25, 331-340.

Conrad, J.T. & Hoover, P. (1982) Variations in the mechanical behaviour of the rabbit cervix with endocrine state and anatomic site. American Journal of Obstetrics and Gynecology, 143, 661-666.

Cross, W.G. & Pitkin, R.M. (1978) Laminaria as an adjunct in induction of labour. Obstetrics and Gynecology, 51, 606-608.

Cullen, B.M. & Harkness, R.D. (1964) Effects of ovariectomy and of

hormones on collagenous framework of the uterus. American Journal of Physiology, 206, 621-627.

Danforth, D.N., Buckingham, J.C. & Roddick, J.W. (1960) Connective tissue changes incident to cervical effacement. American Journal of Obstetrics and Gynecology, 80, 939-945.

Edwards, R.G. & Steptoe, P.C. (1983) Current states of in vitro fertilisation and implantation of human embryos. Lancet, ii, 1265-1269.

Eichner, E., Herman, I., Kritzer, L., Platock, G.M. & Rubinstein, L. (1958) The effects of relaxin on term and premature labour. Annals of the New York Academcy of Sciences, 75, 1023-1032.

Ekman, G. Forman, A., Marsal, K. & Ulmsten (1983) Intravaginal versus intracervical application of prostaglandin E_2 in viscous gel for cervical priming and induction of labour at term in patients with an unfavourable cervical state. American Journal of Obstetrics and Gynecology, 147, 657-661.

Essig, M., Schoenfeld, C., Amelar, R.D., Dubin. L. & Weiss, G. (1982) Stimulation of human sperm motility by relaxin. Fertility and Sterility, 38, 339-343.

Evans, M.I., Dougan, M., Moawad, A.H., Evans, W.J., Bryant-Greenwood, G.D. & Greenwood, F.C. (1983) Ripening of the human cervix with porcine relaxin. American Journal of Obstetrics and Gynecology, 147, 410-414.

Farbrot, E. (1952) The relationship of the effect and pain of pregnancy to the anatomy of the pelvis. Acta Radiology (Stockholm), 38, 403-419.

Fevold, H.L., Hisaw, F.L. & Meyer, R.K. (1930) The relaxative hormone of the corpus luteum. Its purification and concentration. Journal of the American Chemistry Society, 52, 3340-3348.

Fields, P.R., Pardo, R. & Larkin, L.H. (1981) Non-ovarian sources of relaxin. In Relaxin, eds. Greenwood, F.C., Bryant-Greenwood, G.D. & Niall, H.D. pp. 179-185. Amsterdam: Elsevier.

Finn, C.A. (1968) Increase in length of the uterus at the time of implantation in the mouse. Journal Reproduction and Fertility, 7, 69-74.

Finn, C.A. (1977) The implantation reaction. In Biology of the Uterus, ed. Wynn, R.M. pp. 245-308. New York: Plenum Press.

Flynn, A., Kelly, J., Hollins, G. & Lynch, P.F. (1978) Ambulation in labour. British Medical Journal, 2, 591-595.

Frieden, E.H. (1963) Purification and electrophoretic properties of relaxin preparations. Transactions of the New York Academy of Sciences, 25, 331-336.

Frieden, E.H., Rawitch, A.B., Wu, L.C. & Chen, S.C. (1980) The isolation of two proline-containing relaxin species from a porcine relaxin concentrate. Proceedings of the Society of Experimental Biology and Medicine, 163, 521-527.

Gardner, R.L. (1975) Analysis of determination and differentiation in the early mammalian embryo using intra and interspecific chimeras. In The Developmental Biology of Reproduction, eds. Markert, C.L. & Papaconstantinou, J. pp. 207-236. New York: Academic Press.

Gil, J. (1980) Organisation of microcirculation in the lung. Annals of Review Physiology, 42, 177-186.

Gordon, A.J. & Calder, A.A. (1977) Oestradiol applied locally to ripen the unfavourable cervix. Lancet, ii, 1319-1321.

Greenwood, F.C., Mercado-Simmen, R., Yamamato, S., Veno, M. & Bryant-Greenwood, G.D. (1981) Studies on the relaxin receptor. In Relaxin, eds. Greenwood, F.C., Bryant-Greenwood, G.D. & Niall, H.D. pp. 349-356. Amsterdam: Elsevier.

Griss, G., Keck, J., Engelhorn, R. & Tuppy, H. (1967) The isolation and purification of an ovarian polypeptide with uterine-relaxing activity. Biochimica et Biophysica Acta, 140, 45-54.

Hall, K. & Sara, V. (1978) The significance of growth factors for fetal growth. In Abnormal Fetal Growth: Biological Bases and Consequences, ed. Naftolin, F. pp. 121-136. Berlin: Dahlem Konferenzen.

Hisaw, F.L. (1926) Experimental relaxation of the pubic ligament of the guinea pig. Proceedings of the Society for Experimental Biology and Medicine, 23, 661-663.

Hisaw, F.L., Hisaw, F.L. Jr. & Alden, D.B. (1967) Effects of relaxin

on the endothelium of endometrial blood vessels in monkeys (Macaa mulatta). Endocrinology, 81, 375-385.

Hochman, H., Weiss, G., Steinetz, B. & O'Byrne, E. (1978) Serum relaxin concentrations in prostaglandin and oxytocin-induced labour in women. American Journal of Obstetrics and Gynecology, 130, 473-474.

Hollingsworth, M., Gallimore, S. & Isherwood, C.N.M. (1980) Effects of prostaglandins $F_{2\alpha}$ and E_2 on cervical extensibility in the late pregnant rat. Journal of Reproduction and Fertility, 58, 95-99.

Hollingsworth, M., Isherwood, C.N.M. & Foster, R.W. (1979) The effects of oestradiol benzoate, progesterone, relaxin and ovariectomy on cervical extensibility in the late pregnant rat. Journal of Reproduction and Fertility, 56, 471-477.

Hudson, P., Haley, J., John, M., Cronk, M., Crawford, R., Haralambidis, J., Tregear, G., Shine, J. & Niall, H. (1983) Structure of a genomic clone encoding biologically active human relaxin. Nature, 301, 628-631.

James, R., Niall, H., Kwok, S. & Bryant-Greenwood, G. (1977) Primary structure of porcine relaxin; homology with insulin and related growth factors. Nature, 267, 544-546.

John, M.J., Borjesson, B.W., Walsh, J.C. & Niall, H.D. (1981) The amino acid sequence of rat relaxin. In Relaxin, eds. Greenwood, F.C., Bryant-Greenwood, G.D. & Niall, H.D. pp. 37-43. Amsterdam: Elsevier.

Jones, P.A. & Scott-Burden, T. (1979) Activated macrophages digest

extracellular matrix proteins produced by cultured cells. Biochemistry and Biophysics Research, 86, 71-77.

Junqueira, L.C.U., Zugaib, M., Montes, G.S., Toledo, O.M.S., Krisztan, R.M. & Shigihara, K.M. (1980) Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilatation. American Journal of Obstetrics and Gynecology, 138, 273-281.

Kelly, J.V. (1959) Effects of relaxin on the non-pregnant and pregnant uterus. Annals of the New York Academy of Sciences, 75, 998-1002.

Kendall, J.Z., Plopper, C.G. & Bryant-Greenwood, G.D. (1979) Ultrastructural immunoperoxidase demonstration of relaxin in corpora lutea from a pregnant sow. Biology of Reproduction, 18, 94-98.

Kendall, J.Z., Smith, R.G., Webb, P.D., Shih, L.N. & Tate, W.H. (1983) Characterization of prostatic relaxin. In Biology of Relaxin and its Role in the Human, eds. Bigazzi, M., Greenwood, F.C. & Gasparri, F. pp. 363-366. Amsterdam: Excerpta Medica.

Kennedy, T. (1976) Does prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) mediate the effect of relaxin on cervical tone in the rat? Proceedings of the Canadian Federation of Biological Societies, 19, 273-278.

Kerin, J.F., Warnes, L.M. Quinn, P., Kirby, C., Seamark, R.F., Jeffrey, R., Matthews, C.D. & Cox, L.W. (1983) Incidence of multiple pregnancy after in vitro fertilisation and embryo transfer. Lancet, ii, 537-540.

Kertiles, L.P. & Anderson, L.L. (1979) Effect of relaxin on cervical

dilatation, parturition and lactation in the pig. Biology of Reproduction, 21, 57-68.

Klopper, A. (1983) The first days. British Journal of Obstetrics and Gynaecology, 90, 593-594.

Koay, E.S.C., Too, C.K.L., Greenwood, F.C. & Bryant-Greenwood, G.D. (1983) Relaxin stimulates collagenase and plasminogen activator secretion by dispersed human amnion and chorion cells in vitro. Journal of Clinical Endocrinology and Metabolism, 56, 1332-1334.

Krantz, J.C., Bryant, H.H. & Carr, C.J. (1950) The action of aqueous corpus luteum extract upon uterine activity. Surgery Gynecology and Obstetrics, 90, 372-375.

Kupperman, H.S., Rosenberg, D. & Cutler, A. (1958) Relaxin in dysmenorrhoea and its effect in vitro upon muscular contraction. Annals of New York Academy of Science, 75, 1003-1010.

Kwok, S.C., Chamley, W.A. & Bryant-Greenwood, G.D. (1978) High molecular weight forms of relaxin in pregnant sow ovaries. Biochemical Biophysical Research Communications, 82, 997-1005.

Kwok, S.C.M., Bryant-Greenwood, G.D. & Niall, H.D. (1980) Evidence for proteolysis during purification of relaxin from pregnant sow ovaries. Endocrinology Research Communications, 7, 1-12.

Larkin, L.H., Pardo, R.J. & Renegar, R.H. (1983) Sources of relaxin and morphology of relaxin containing cells. In Biology of Relaxin and its Role in the Human, eds. Bigazzi, M., Greenwood, F.C. & Gasparri,

F. pp. 191-205. Amsterdam: Excerpta Medica.

Lenton, E.A., Salaiman, R., Wobowale, O. & Cooke, D. (1982) The human menstrual cycle: plasma concentration of prolactin, LH, FSH, oestradiol and progesterone in conceiving and non-conceiving women. Journal of Reproduction and Fertility, 65, 131-139.

Leppi, T.J. & Kinnison, P.A. (1971) The connective tissue ground substance in the mouse uterine cervix: an electron microscopic histochemical study. Anatomical Records, 170, 97-118.

Liggins, G.C. (1981) Cervical ripening as an inflammatory reaction. In The Cervix in Pregnancy and Labour, eds. Ellwood, D.A. & Anderson, A.B.M. p. 1. Edinburgh: Churchill Livingstone.

Liggins, G.C. (1984) Current concepts in the initiation of labour. Abstract in The Proceedings of the Endocrine Physiology of Pregnancy and Peripartal Period. p. 43. Siena: Serono-Symposia.

Loumaye, E., Decooman, S. & Thomas, K. (1980) Immunoreactive relaxin-like substance in human seminal plasma. Journal of Clinical Endocrinology and Metabolism, 50, 1142, 1143.

Lutjen, P.J., Findlay, J., Trounson, A.O., Leeton, J., Burger, H.G. & Hudson, B. (1983) The effects of cyclic steroid replacement on plasma steroids and gonadotrophins in patients with premature ovarian failure. Proceedings of the Endocrine Society of Australia, 26, 112-116.

MacKenzie, I.Z. & Embrey, M.P. (1977) Cervical ripening with intravaginal prostaglandin E_2 gel. British Medical Journal, 2, 1381-1384.

MacKenzie, I.Z. & Embrey, M.P. (1978) The influence of pre-induction vaginal prostaglandin E_2 gel upon subsequent labour. British Journal of Obstetrics and Gynaecology, 85, 657-661.

MacKenzie, I.Z. & Embrey, M.P. (1979) A comparison of PGE_2 and $PGF_{2\alpha}$ vaginal gel for ripening the cervix before induction of labour. British Journal of Obstetrics and Gynaecology, 86, 167-170.

MacLennan, A.H. (1981) Cervical ripening and the induction of labour by vaginal prostaglandin $F_{2\alpha}$ and relaxin. The cervix in pregnancy and labour. In Clinical and Biochemical Investigations, eds. Ellwood, D. & Anderson, A. pp. 187-195. Edinburgh: Churchill Livingstone.

MacLennan, A.H. (1981) Relaxin - a review. Australian and New Zealand Journal of Obstetrics and Gynaecology, 21, 195-202.

MacLennan, A.H. (1983) The role of relaxin in human reproduction. Clinical Reproduction and Fertility, 2, 77-95.

MacLennan, A.H. & Green, R.C. (1979) Cervical ripening and induction of labour with intravaginal prostaglandin $F_{2\alpha}$. Lancet, i, 117-119.

MacLennan, A.H. & Green, R.C. (1980) A double-blind dose trial of intravaginal prostaglandin $F_{2\alpha}$ for cervical ripening and the induction of labour. Australian and New Zealand Journal of Obstetrics and Gynaecology, 20, 80-83.

MacLennan, A.H., Bryant-Greenwood, G.D. & Greenwood, F.C. (1983) Clinical studies with porcine relaxin. In Biology of Relaxin and its Role in the Human, eds. Bigazzi, M., Greenwood, F.C. & Gasparri, F. p.

329. Amsterdam: Excerpta Medica.

MacLennan, A.H., Green, R.C., Bryant-Greenwood, G.D., Greenwood, F.C. & Seamark, R.F. (1980) Ripening of the human cervix and induction of labour with purified porcine relaxin. Lancet, i, 220-223.

MacLennan, A.H., Green, R.C., Bryant-Greenwood, G.D., Greenwood, F.C. & Seamark, R.F. (1981) Cervical ripening with combinations of vaginal prostaglandin $F_{2\alpha}$, oestradiol and relaxin. Obstetrics and Gynecology, 58, 601-604.

MacLennan, A.H., Katz, M. & Creasy, R. (1985) The morphology of cervical ripening induced by the hormones relaxin and prostaglandin $F_{2\alpha}$ in a rabbit model. American Journal of Obstetrics and Gynecology, (in press).

MacLennan, A.H., Nicolson, R. & Green, R.C. (1985) Serum relaxin levels in normal human pregnancy. Australian and New Zealand Journal of Obstetrics and Gynaecology, (submitted to).

Martin, P.A., Sherwood, O.D. & Dziuk, D.J. (1979) Effect of CL number on relaxin levels in pregnant pigs. Biology of Reproduction, 20, 81-86.

McCarthy, J.J., Erving, H.W. & Lauffe, E.L. (1957) Preliminary report on the use of relaxin in the management of threatened premature labour. American Journal of Obstetrics and Gynecology, 74, 134-138.

McLaren, A. & Michie, D. (1959) The spacing of implantations in the mouse uterus. Memoranda of the Society of Endocrinology, 6, 67-75.

McMurtry, J.P., Kwok, S.C.M. & Bryant-Greenwood, G.D. (1979) Target tissues for relaxin identified in vitro with ^{125}I-labelled porcine relaxin. Journal of Reproduction and Fertility, 53, 209-216.

McMurtry, J.P., Floershein, G.L. & Bryant-Greenwood, G.D. (1980) Characterisation of the binding of ^{125}I-labelled succinylated porcine relaxin to human and mouse fibroblasts. Journal of Reproduction and Fertility, 58, 43-49.

Mellows, J.H., Sims, C.D. & Craft, I. (1977) Prostaglandin E_2 in tylose for induction of labour in patients with a favourable cervix. Proceedings of the Royal Society of Medicine, 70, 537-540.

Mercado, R.C., Bryant-Greenwood, G.D. & Greenwood, F.C. (1980) Characterisation of the binding of ^{125}I-relaxin to rat uterus. Journal of Biology and Chemistry, 255, 3617-3623.

Mercado-Simmen, R.C., Bryant-Greenwood, G.D. & Greenwood, F.C. (1982) Relaxin receptor in the rat myometrium: regulation by estrogen and relaxin. Endocrinology 110, 220-226.

Miller, J.W., Kisley, A. & Murray, W.J. (1957) The effects of relaxin-containing ovarian extracts on various types of smooth muscle. Journal of Pharmacology and Experimental Therapeutics, 120, 426-437.

Mitchell, M.D., Flint, A.P.F., Bibby, J., Brunt, J., Arnold, J.M., Anderson, A.B.M. & Turnbull, A.C. (1977) Rapid increases in plasma prostaglandin concentrations after vaginal examintion and amniotomy. British Medical Journal, 2, 1183-1185.

Niall, H.D. (1979) Relaxin. An insulin-related growth factor. Progress in Clinical and Biological Research, 31, 651-658.

Nishikori, K., Weisbrodt, N.W., Sherwood, O.D. & Sanborn, B.M. (1983) Effects of relaxin on rat uterine myosin light chain kinase activity and myosin light chain phosphorylation. Journal of Biology and Chemistry, 258, 2468-2476.

Nissenson, R., Flouret, G. & Hetchter, O. (1978) Opposing effects of estradiol and progesterone on oxytocin receptors in the rabbit uterus. Proceedings of the National Academy of Science United States of America, 75, 2044-2048.

Norström, A. and Wiqvist, I. Relaxin-induced changes in adenosine 3¹, 5¹-monophosphate levels in the human cervix. (In press).

Norström, A., Wiqvist, I., Hamberger, L. & Karlsson, I. (1983) Cyclic adenosine monophosphate as a factor in prostaglandin-mediated influence on collagen synthesis in the human uterine cervix. In Advances in Prostaglandin, Thromboxane and Leukotriene Research, eds. Samuelsson, B., Paoletti, R. & Ramwell, P. Ch. 12, p 465. New York: Raven Press.

Norström, A., Bryman, I., Wiqvist, N., Sabni, S. & Lindblom, B. (1984) Inhibitory action of relaxin on human cervical smooth muscle. Journal of Clinical Endocrinology and Metabolism, 59, 379-382.

O'Byrne, E.M. & Steinetz, B.G. (1976) Radioimmunoassay (RIA) of relaxin in sera of various species using an antiserum to porcine relaxin. Proceedings of the Society of Experimental Biology and

Medicine, 152, 272-276.

O'Byrne, E.M., Carriere, B.T., Sorensen, L., Segaloff, A., Schwabe, C. & Steinetz, B.G. (1978) Plasma immunoreactive relaxin levels in pregnant and non-pregnant women. Journal of Clinical Endocrinology and Metabolism, 47, 1106-1110.

O'Byrne, E.M., Flitcraft, J.F., Sawyer, W.K., Hochman, J., Weiss, G. & Steinetz, B.G. (1978) Relaxin bioactivity and immunoactivity in human corpora lutea. Endocrinology, 102, 1641-1644.

O'Byrne, E.M., Butler, M., Hickman, L., Quintavalla, J., Nusynowitz, I. & Steinetz, B.G. (1982) Histochemical and morphological changes in the mouse pubic joint during oestrogen priming and in response to relaxin. Annals of New York Academy of Science, 380, 217- .

Parry, D.M. & Ellwood, D.A. (1981) Ultrastructural aspects of cervical softening in the sheep. In The Cervix in Pregnancy and Labour, eds. Ellwood, D.A. & Anderson, A.B.M. p. 74. Edinburgh: Churchill Livingstone.

Porter, D.G. (1979a) The myometrium and the relaxin enigma. Animal Reproduction Science, 2, 77-96.

Porter, D.G. (1979b) Relaxin: old hormone, new prospect. In Oxford Reviews of Reproductive Biology, ed. Finn, C.A. pp 1-57. Oxford: Clarendon Press.

Porter, D.G., Downing, S.J. & Bradshaw, J.M.C. (1979) Relaxin inhibits spontaneous and prostaglandin-driven myometrial activity in

anaesthetised rats. Journal of Endocrinology, 83, 183-192.

Psychoyos, A. (1973) Endocrine control of egg implantation. In Handbook of Physiology, ed. Greep, R.O. Vol. II p 197. American Physiological Society.

Pusey, J., Kelly, W.A., Bradshaw, J.M.C. & Porter, D.G. (1980) Myometrial activity and the distribution of the blastocysts in the uterus of the rat: interference by relaxin. Biology of Reproduction, 23, 394-397.

Quagliarello, J., Nachtigall, R., Goldsmith, L.T., Hochman, J., Steinetz, B.G., O'Byrne, E.M. & Weiss, G. (1979) Serum immunoreactive relaxin concentrations in human pregnancy, labour and the puerperium. Advances in Experimental Medicine and Biology, 112, 743-748.

Quagliarello, J., Szlachter, N., Steinetz, B.G., Goldsmith, L. & Weiss, G. (1979) Serial relaxin concentrations in human pregnancy. American Journal of Obstetrics and Gynaecology, 135, 43-44.

Quagliarello, J., Goldsmith, L., Steinetz, B., Lustig, D.S. & Weiss, G. (1980) Induction of relaxin secretion in non-pregnant women by human chorionic gonadotrophin. Journal of Clinical Endocrinology and Metabolism, 51, 74-77.

Rodbard, D., Bridson, W. & Rayford, P.L. (1969) Rapid calculation of radioimmunoassay results. Journal of Laboratory and Clinical Medicine, 74, 770-781.

Rogers, P.A.W., Murphy, C.R. & Gannon, B.J. (1982) Changes in the

spatial organization of the uterine vasculature during implantation in the rat. Journal of Reproduction and Fertility, 65, 211-214.

Rogers, P.A.W., Murphy, C.R., Squires, K.R. & MacLennan, A.H. (1983) Effects of relaxin on the intrauterine distribution and antimesometrial positioning and orientation of rat blastocysts before implantation. Journal of Reproduction and Fertility, 68, 431-435.

Sarosi, P., Schmidt, C.L., Essig, M., Steinetz, B.G. & Weiss, G. (1983) The effect of relaxin and progesterone on rat uterine contractions. American Journal of Obstetrics and Gynecology, 145, 402-405.

Sarosi, P., Schoenfeld, C., Berman, J., Basch, R., Amelar, R.D., Dubin, L., Steinetz, B.G. & Weiss, G. (1983) The effect of rabbit anti-porcine relaxin antibody on sperm motility in vitro. Abstracts of the Annual Meeting of the American Fertility Society. Fertility and Sterility, 39, 421.

Sawyer, W.H., Frieden, E.H. & Martin, A.C. (1953) In vitro inhibition of spontaneous contractions of the rat uterus by relaxin-containing extracts of sow ovaries. American Journal of Physiology, 172, 547-552.

Scarselli, G., Bigazzi, M., Acanfora, L., Cozzi, C., Branconi, F. & Nardi, M. (1983) RIA assay of relaxin in human pregnancy. In Biology of Relaxin and its Role in the Human, eds. Bigazzi, M., Greenwood, F.C. & Gasparri, F. pp 318-320. Amsterdam: Excerpta Medica.

Schwabe, C. (1983) N-α-formyl-tyrosyl-relaxin - a reliable tracer for relaxin radioimmunoassay. Endocrinology, 113, 814-815.

Schwabe, C. & Harmon, S.J. (1978) A comparative circular dichroism study of relaxin and insulin. Biochemical Biophysical Research Communications, 84, 374-380.

Schwabe, C., McDonald, J.K. & Steinetz, B.G. (1976) Primary structure of the A chain of porcine relaxin. Biochemical Biophysical Research Communications, 70, 397-405.

Schwabe, C., McDonald, J.K. & Steinetz, B.G. (1977) Primary structure of the B chain of porcine relaxin. Biochemical Biophysical Research Communications, 75, 503-510.

Schwabe, C., Steinetz, B., Weiss, G., Segaloff, A., McDonald, J.K., O'Byrne, E., Hochman, J., Carriere, B. & Goldsmith, L. (1978) Relaxin Recent Progress in Hormonal Research, 34, 123-211.

Sherwood, O.D. (1979) Purification and characterization of rat relaxin. Endocrinology, 104, 886-892.

Sherwood, O.D., Chang, C.C., DeVier, G.W., Diehl, J.R. & Dziuk, P.J. (1976) Relaxin concentrations in pig plasma following the administration of prostaglandin $F_2\alpha$ during late pregnancy. Endocrinology, 98, 875-879.

Sherwood, O.D. & O'Byrne, E.M. (1974) Purification and characterisation of porcine relaxin. Archives of Biochemistry and Biophysics, 160, 185-196.

Sherwood, O.D., Rosentreter, K.R. & Birhimer, M.D. (1975) Development of a radioimmunoassay for porcine relaxin using ^{125}I-labelled poly-

tyrosyl-relaxin. Endocrinology, 96, 1106-1113.

Steiner, A.L. & Creasy, R.K. (1983) Methods of cervical priming. Clinical Obstetrics and Gynecology, 26, 37-46.

Steinetz, B.G., Beach, V.L. & Kroc, R.L. (1959) In Methods in Hormone Research, ed. Dorfman, R. 2nd ed. Vol 2A pp 481-513. New York: Academic Press.

Steinetz, B., Beach, V.L., Kroc, R.L. Stassili, N.R., Nussbaum, R.E., Nemith, P.J. & Dunn, R.K. (1960) Bioassay of relaxin using a reference standard: a simple and reliable method utilizing direct measurement of intrapubic ligament formation in mice. Endocrinology, 67, 102-115.

Steinetz, B.G., O'Byrne, E.M. & Kroc, R.L. (1980) The role of relaxin in cervical softening during pregnancy in mammals. In Dilatation of the Uterine Cervix, eds., Naftolin, F. & Stubblefield, P.G. pp 157-177. New York: Raven Press.

St. Louis, J. (1981) Relaxin inhibition of KCl-induced uterine contractions in vitro: an alternative bioassay. Canadian Journal of Physiology and Pharmacology, 59, 507-512.

Stone, M.L., Sedlis, A. & Zuckerman, M.B. (1959) Effects of relaxin on term and premature labour. Annals of the New York Academy of Science, 75, 1011-1015.

Szlachter, N., O'Byrne, E., Goldsmith, L., Steinetz, B.G. & Weiss, G. (1980) Myometrial inhibiting activity of relaxin-containing extracts

of human corpora lutea of pregnancy. American Journal of Obstetrics and Gynecology, 136, 584-586.

Szlachter, B.N., Quagliarello, J., Jewelewicz, R., Osathanodh, R. Spellacy, W.N. & Weiss, G. (1982) Relaxin in normal and pathogenic pregnancies. Obstetrics and Gynecology, 59, 167-170.

Thomas, K., Loumaye, E. and Ferin, J. (1980) Relaxin in non-pregnant women during ovulation stimulation. Gynecological and Obstetrical Investigation, 11, 75-80.

Turnbull, A.C. & Anderson, A.B.M. (1968) Induction of labour. Journal of Obstetrics and Gynaecology of the British Commonwealth, 75, 24-41.

Uldbjerg, N., Edman, G., Malmstrøm, A., Sporrong, B., Ulmsten, U. & Wingerup, L. (1981) Biochemical and morphological changes of human cervix after local application of prostaglandin E_2 in pregnancy. Lancet, i, 267-268.

Vasilenko, P., Frieden, E.H. & Adams, W.C. (1980) Effect of purified relaxin on uterine glycogen and protein in the rat. Proceedings of the Society of Experimental Biology and Medicine, 163, 245-248.

Von Maillot K., Weib, B., Nagelschmidt, M. & Struk, H. (1977) Muttermundseroffnung und relaxin. Archives of Gynecology, 223, 323-331.

Walsh, J.R. & Niall, H.D. (1980) The use of octadecylsilica purification method minimizes proteolysis during isolation of porcine and rat relaxins. Endocrinology, 107, 1258-1260.

Weib, M., Magelschmidt, M. & Struk, H. (1979) Relaxin and collagen metabolism. Hormone and Metabolic Research, 11, 408-410.

Weiss, G. (1984) In post paper discussion on the effect of in vivo progesterone administration on relaxin-inhibited rat uterine contractions. American Journal of Obstetrics and Gynecology, 148, 946-950.

Weiss, G., O'Byrne, E.M. & Steinetz, B.G. (1976) Relaxin: a product of the human corpus luteum of pregnancy. Science, 194, 949-949.

Weiss, G., O'Byrne, E.M., Hochman, J.A., Goldsmith, L.T., Rifkin, I. & Steinetz, B.G. (1977) Secretion of progesterone and relaxin by the human corpus luteum at midpregnancy and at term. Obstetrics and Gynecology, 50, 679-681.

Weiss, G., O'Byrne, E.M., Hochman, J., Steinetz, B.G., Goldsmith, L. & Flitcraft, I.G. (1978) Distribution of relaxin in women during pregnancy. Obstetrics and Gynecology, 52, 569-570.

Wiqvist, N. (1959) The effect of prolonged administration of relaxin on some functional properties of the non-pregnant mouse and rat uterus. Acta Endocrinology Supplement, 46, 15-32.

Wiqvist, N. & Paul, K.G. (1958) Inhibition of the spontaneous uterine motility in vitro as bioassay of relaxin. Acta Endocrinology, 29, 135-146.

Wilkinson, J.A. (1963) Prime factors in the etiology of congenital dislocation of the hip. Journal of Bone and Joint Surgery, 45B, 268-283.

Wingerup, L., Anderson, K. & Ulmstem, U. (1979) Ripening of the cervix and induction of labour in patients at term by single intra-cervical application of prostaglandin E_2 in viscous gel. Acta Obstetrics and Gynecology Scandanavia (Suppl), 84, 11-14.

Zarrow, M.X. & McClintock, J.A. (1966) Localization of ^{131}I-labelled antibody to relaxin. Journal of Endocrinology, 36, 377-387.

Zarrow, M.X., Neher, G.M., Sikes, D., Brennan, D.M. & Bullard, J.F. (1956) Dilation of the uterine cervix of the sow following treatment with relaxin. American Journal of Obstetrics and Gynecology, 72, 260-264.