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RELAXIN AT PARTURITION IN THE PIG

O.D. SHERWOOD
School of Basic Medical Sciences and Department of Physiology and Biophysics, University of Illinois, USA

Relaxin is a polypeptide hormone which is found in the blood and reproductive organs during pregnancy in many species (Schwabe *et al.*, 1978; Porter, 1979b). The hormone was discovered in 1926 when Hisaw found that injection of serum obtained from pregnant rabbits or pregnant guinea pigs into adult female guinea pigs at oestrus caused a relaxation of the pubic symphysis which was similar to that which occurs during late pregnancy (Hisaw, 1926). Within three years of the discovery of these hormonal effects, Hisaw (1929) reported that the relaxative hormone was also present in the blood of pregnant sows and that sow corpora lutea were a 'very excellent source for the substance'. A crude aqueous extract of the hormone was first obtained from pregnant sow ovaries by Fevold, Hisaw and Meyer (1930), and they named the hormone 'relaxin'. From 1930 until the present, most of our knowledge about the chemistry and physiology of relaxin has been learned from studies which employed porcine relaxin.

This chapter will be largely confined to the physiology of relaxin at parturition in the pig. For more comprehensive descriptions of the chemistry of relaxin and physiological studies of relaxin in species other than the pig, the reader is referred to the following recent reviews: Schwabe *et al.* (1978), Porter (1979b), Steinetz, O'Byrne and Kroc (1980), Niall *et al.* (1982) and Sherwood (1982a,b).

Isolation and chemistry of porcine relaxin

Fevold, Hisaw and Meyer (1930) first showed that an aqueous extract of sow corpora lutea contained relaxin, and they concluded that relaxin might be a peptide-like molecule since it was amphoteric and vulnerable to trypsin digestion. Nearly all subsequent efforts to isolate relaxin employed ovaries obtained from pigs during late pregnancy, since this source of the hormone was known to have a high content of relaxin activity (Hisaw and Zarrow, 1948) and was relatively easy to acquire in large quantities. Progress toward the isolation of porcine relaxin was slow. Well documented progress toward the purification of relaxin was first made in the 1950s and 1960s, and this progress was largely attributable to the availability of new techniques for the isolation and characterization of proteins, as well as new or improved methods for the bioassay of relaxin. The relaxin bioassays

most commonly used to monitor relaxin isolation efforts involved either *in vivo* stimulation of interpubic ligament formation in oestrogen-treated mice or guinea pigs, or *in vitro* inhibition of spontaneous contractions of uteri obtained from oestrogen-treated rats (Steinetz, Beach and Kroc, 1969). During the 1960s three laboratories reported procedures whereby they obtained preparations of porcine relaxin which contained high biological activity (Cohen, 1963; Frieden, Stone and Layman, 1960; Griss *et al.*, 1967). Although the homogeneity of these relaxin preparations was not rigorously established and the physicochemical properties of porcine relaxin were not precisely described, it is important to appreciate that these workers correctly indicated that porcine relaxin is a protein with a molecular weight between 4000 and 10 000 (Frieden, Stone and Layman, 1960; Cohen, 1963; Griss *et al.*, 1967), that it has a basic isoelectric point (Cohen, 1963; Griss *et al.*, 1967), and that it contains disulphide bonds which are essential for biological activity (Frieden and Hisaw, 1953; Cohen, 1963).

In 1974 we reported (Sherwood and O'Byrne, 1974) a simple isolation procedure whereby three essentially homogeneous preparations of porcine relaxin, designated CMB, CMa, and CMa′, are obtained in quantities sufficient for detailed chemical and physiological studies (*Figure 17.1*). Physicochemical studies indicated that the three porcine relaxin preparations are nearly identical to one another. They have molecular weights of approximately 6000, nearly identical amino acid compositions, contain no histidine, proline, or tyrosine, and consist of two chains of similar size, designated A and B, which are linked by disulphide bonds (Sherwood and

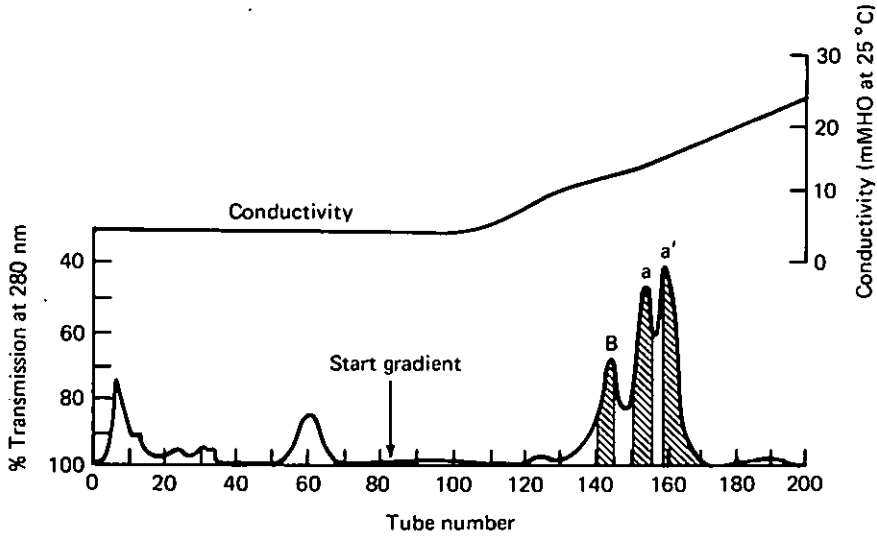

Figure 17.1 The final step in the isolation of porcine relaxin. Partially purified porcine relaxin was adsorbed to a column of carboxymethylcellulose. A linear gradient, which consisted of the column equilibrating buffer plus NaCl, brought about the elution of three contiguous peaks which contained relaxin activity. The contents of the tubes denoted by hatching were pooled to form the three highly purified relaxin preparations CMB, CMa, and CMa′. From Sherwood and O'Byrne (1974)

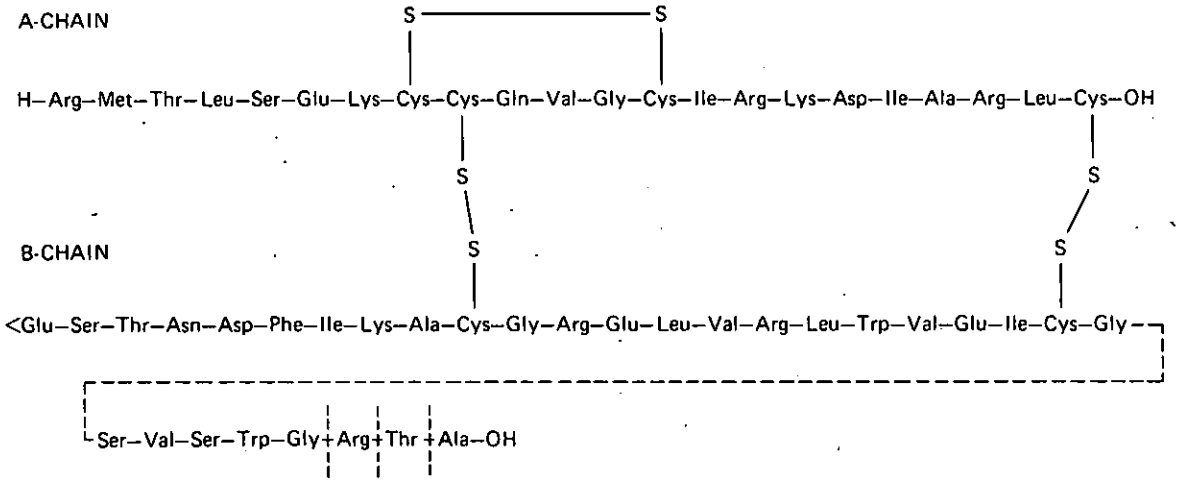

Figure 17.2 The primary structure of porcine relaxin reported by Niall *et al.* (1981). These workers attribute the multiple forms of porcine relaxin to differences among their B chains which range from 28–31 amino acids as shown in the figure above. The N-terminal amino acid in the B chain is pyroglutamic acid. The position of the disulphide linkages are as reported by Schwabe and McDonald (1977). Small differences remain in the amino acid sequences which have been reported for porcine relaxin. Schwabe, McDonald and Steinetz (1976, 1977) have reported that the A chain contains glutamic acid rather than glutamine in position 10, and that the B chain contains only 26 amino acids with C-terminal sequence Ile-Cys-Gly-Val-Trp-Ser

O'Byrne, 1974). We also reported that porcine relaxin preparation CMa has 22 amino acids in the A chain and 28–31 amino acids in the B chain. Two groups (Schwabe, McDonald and Steinetz, 1976, 1977; James *et al.*, 1977; Niall *et al.*, 1981) have determined the amino acid sequences of the A and B chains of porcine relaxin (*Figure 17.2*). Niall and his coworkers recently reported that the multiple forms of porcine relaxin are attributable to slight differences in the lengths of B chains which may result from varying degrees of proteolysis of the C-terminus during the isolation procedure (Walsh and Niall, 1980; Niall *et al.*, 1982).

Relaxin has structural features which are similar to those of insulin, and it has been suggested that relaxin and insulin may have evolved from a common ancestral gene (Schwabe and McDonald, 1977; James *et al.*, 1977). Schwabe and McDonald (1977) showed that the locations of the disulphide bonds within porcine relaxin are homologous to those of insulin (*Figure 17.2*). When the disulphide bonds (half-cysteines) of porcine relaxin and porcine insulin are aligned in register, only five residues in addition to the half-cysteines are identical in the two hormones. However, in many cases the amino acid residues which are in comparable positions in the two hormones have similar structures, i.e. are 'conservative substitutions' (Schwabe and McDonald, 1977; James *et al.*, 1977); and it has been suggested on the basis of studies which employed model building (Bedarkar *et al.*, 1977) and computer graphics (Isaacs *et al.*, 1978) that the aminoacid sequence of porcine relaxin permits the hormone to adopt a three-dimensional conformation similar to that of insulin. However, if relaxin and insulin evolved from a common ancestral gene, the following observations seem to indicate that considerable evolutionary divergence has occurred between the two hormones. There is evidence that the biosynthetic precursors for relaxin are larger than those for insulin. Whereas pre-proinsulin and proinsulin have molecular weights of 12 000 and 9000 daltons respectively, putative relaxin precursors with molecular weights of 10 000, 13 000, 19 000 (Kwok, Chamley and Bryant-Greenwood, 1978) and 42 000 daltons (Frieden and Yeh, 1977), which could be converted into about 6000 dalton relaxin, have been reported. Recently, Gast *et al.* (1980) demonstrated in a cell-free system that porcine luteal mRNA directs the synthesis of a protein which is immunologically related to relaxin. This 'relaxin-containing protein' has an apparent molecular weight of approximately 23 000.

Additionally, studies which employed radioimmunoassays for porcine relaxin (Sherwood, Rosentreter and Birkhimer, 1975a) and porcine insulin (Rawitch, Moore and Frieden, 1980) showed no apparent homology between the antigenic determinants of relaxin and insulin. Finally, there is evidence that relaxin does not bind to insulin receptors since highly purified porcine relaxin failed to compete with radioiodinated porcine insulin for insulin receptors on mononuclear leukocytes (Rawitch, Moore and Frieden, 1980).

Relaxin levels in the corpora lutea throughout pregnancy and at parturition

During the luteal phase of the oestrous cycle, relaxin biological activity within pig ovaries is detectable but extremely low compared with the

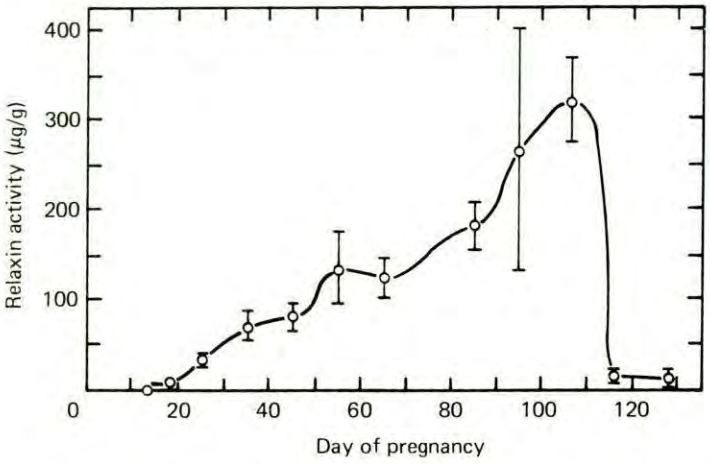

Figure 17.3 Relaxin activity in extracts of porcine corpora lutea obtained throughout pregnancy. Relaxin biological activity was determined by the mouse interpubic ligament bioassay (Steinetz, Beach and Kroc, 1969). The biological activity is expressed in μg of a partially purified NIH porcine relaxin preparation per gram of luteal tissue. From Anderson *et al.* (1973)

relaxin levels present within the ovaries during most of pregnancy (Hisaw and Zarrow, 1948; Anderson *et al.*, 1973; Sherwood and Rutherford, 1981). Hisaw and Zarrow (1948) reported that the amount of relaxin biological activity in pig ovaries was low during early pregnancy and increased approximately 20-fold to maximal concentrations by the time the foetuses attained a length of 5 or 6 inches. More recently, Anderson *et al.* (1973) reported that the levels of relaxin biological activity within corpora lutea rose steadily from day 20 of pregnancy to maximal levels between days 110 to 115 and then declined rapidly within 16 hours of parturition (*Figure 17.3*). Ultrastructural studies of the corpora lutea throughout pregnancy by Belt *et al.* (1971) have also contributed much to an understanding of the synthesis, storage and release of relaxin during pregnancy in the pig. These workers indicated that the most evident change of fine structure of the granulosa lutein cells during pregnancy was in the population of dense membrane-limited granules. These granules, which were not apparent during the oestrous cycle (Cavazos *et al.*, 1969), became a conspicuous constituent of the cytoplasm by day 28, were maximal at days 105 to 110 (*Figure 17.4*), and declined markedly during the 24 hours before parturition (*Figure 17.5*). The appearance, accumulation, and disappearance of these granules during pregnancy closely paralleled the concentrations of relaxin bioactivity in the corpora lutea (*Figure 17.3*) and it was suggested that the cytoplasmic granules are storage sites for relaxin (Belt *et al.*, 1971; Anderson *et al.*, 1973). This view has been strengthened by recent immunocytochemical studies at the ultrastructural level which showed that relaxin immunoactivity is associated with the dense granules which occur in the corpora lutea of pregnant pigs (Corteel, Lemon and Dubois, 1977; Kendall, Plopper and Bryant-Greenwood, 1978).

The regulation of relaxin synthesis and storage in pig corpora lutea is not well understood. Anderson *et al.* (1973) reported that pig corpora lutea

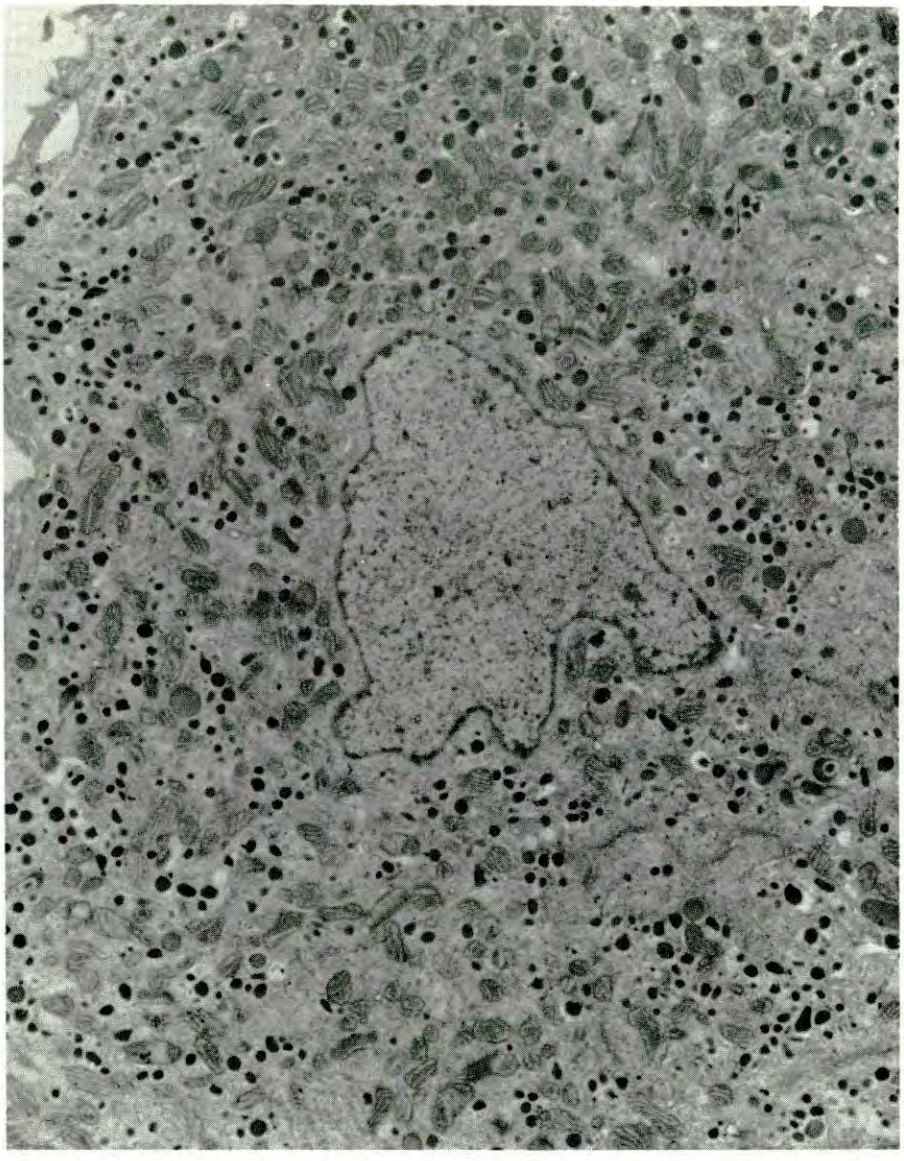

Figure 17.4 A portion of a porcine granulosa lutein cell at day 110. (×6800, reduced to two-thirds in reproduction). The granule content is maximal and the Golgi apparatus is well developed. From Belt *et al.* (1971)

maintained beyond 100 days by hysterectomy or administration of oestrogen during the oestrous cycle contained relaxin bioactivity levels which approached those observed in pregnant pigs. The observation that relaxin levels increased as the age of the corpora lutea increased led these workers to conclude that relaxin levels in pig corpora lutea may be an indication of an ageing process (Anderson *et al.*, 1973).

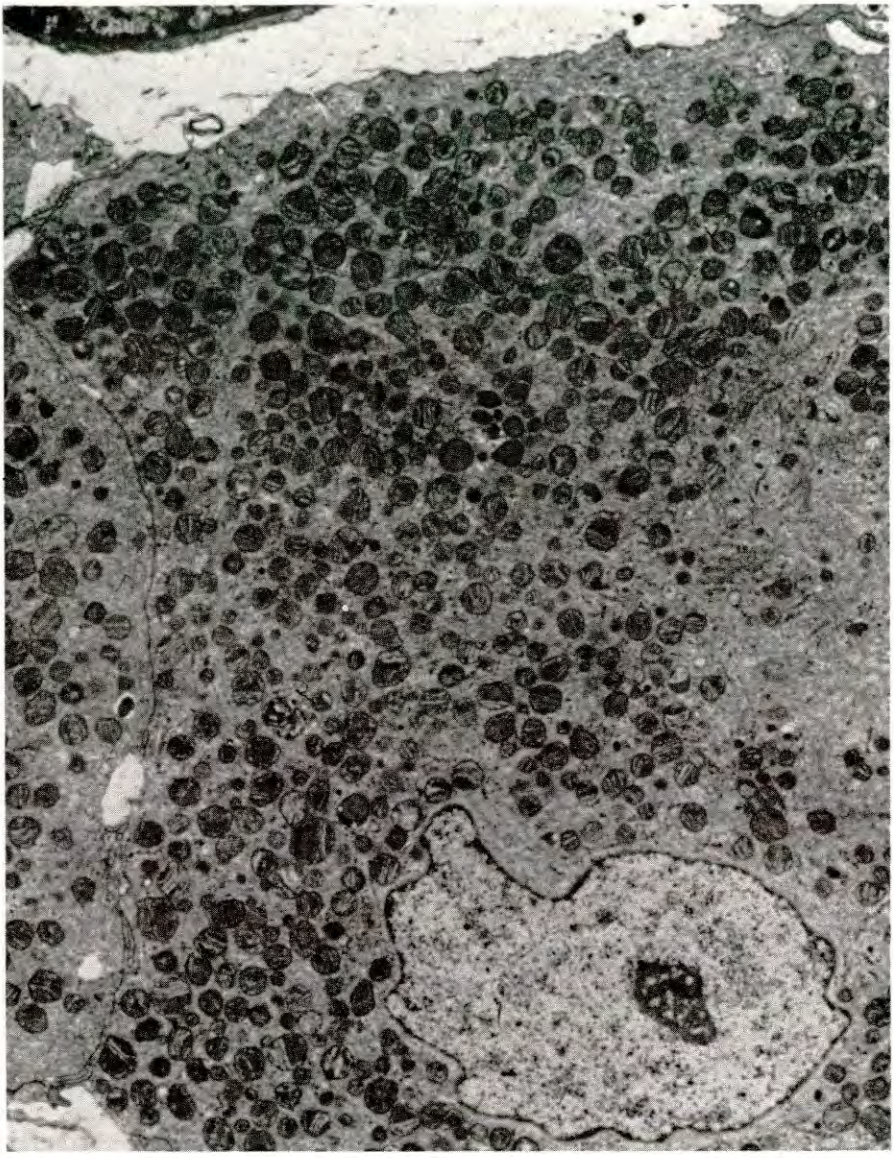

Figure 17.5 A portion of a porcine granulosa lutein cell at day 116, 6 hours prior to parturition (×8500, reduced to two-thirds in reproduction). The granule depletion of the cell is nearly total. From Belt *et al.* (1971)

Relaxin may be produced in ovarian sites other than the corpora lutea in the pig. Anderson *et al.* (1973) reported that extremely low levels of relaxin bioactivity were found in follicular and interstitial tissue of ovaries removed from pregnant pigs and suggested that the relaxin in these ovarian components may result from the transport of the hormone from adjacent corpora lutea. In contrast, an immunofluorescent study of the ovaries of

pregnant pigs which employed an antiserum to highly purified porcine relaxin demonstrated that relaxin immunoactivity was confined to the corpora lutea (Larkin, Fields and Oliver, 1977). Recently, small amounts of relaxin immunoactivity were reported to be present in ovarian follicular fluid obtained from non-pregnant and pregnant sows and also in ovaries of immature sows (Bryant-Greenwood *et al.*, 1980; 1981; Matsumoto and Chamley, 1980).

Relaxin levels in the blood throughout pregnancy and at parturition

BIOASSAY

Efforts to measure the levels of relaxin in peripheral blood were long hindered by the insensitivity and imprecision of relaxin bioassays. Nevertheless, by concentrating ovarian venous blood, Belt *et al.* (1971) demonstrated a sharp increase in relaxin bioactivity in pig plasma between 44 and 26 hours before parturition, i.e. concomitant with the rapid degranulation of granulosa lutein cells in the corpora lutea.

RADIOIMMUNOASSAY

The advent of the radioimmunoassay in the 1960s and the isolation of highly purified porcine relaxin in the mid 1970s (Sherwood and O'Byrne, 1974) made possible the development of assays which are specific for relaxin and sufficiently sensitive to measure the levels of relaxin found in the peripheral blood. In 1975 Sherwood, Rosentreter and Birkhimer (1975) reported the development of a homologous radioimmunoassay for porcine relaxin which routinely detects as little as 32 pg of porcine relaxin and is more than 1000 times more sensitive than the commonly employed mouse interpubic ligament bioassay.

The levels of relaxin in the peripheral blood of pigs throughout gestation and at parturition have been determined with this radioimmunoassay. Relaxin immunoactivity concentrations in peripheral plasma remain below 2 ng/ml until about day 100 and then rise gradually to approximately 5 ng/ml on day 110 (Sherwood *et al.*, 1975). From day 110 to day 112, mean relaxin concentrations increase to approximately 15 ng/ml, and in most pigs small surges in relaxin levels occur during these three days (*Figure 17.6*). During the two days which precede parturition, relaxin levels increase markedly and attain maximal concentrations which generally range from 60 to 250 ng/ml (*Figure 17.6*; Sherwood *et al.*, 1975, 1976, 1978, 1979, 1981). In nearly all cases, this pre-partum elevation in relaxin consists of two or three sustained surges which last for 10–20 hours. The peak which immediately precedes parturition has maximal relaxin concentrations and generally occurs from 14–22 hours before delivery (Sherwood *et al.*, 1975, 1976, 1978, 1979, 1981). This maximum is followed by a decline in relaxin concentrations which does not appear to be interrupted during parturition

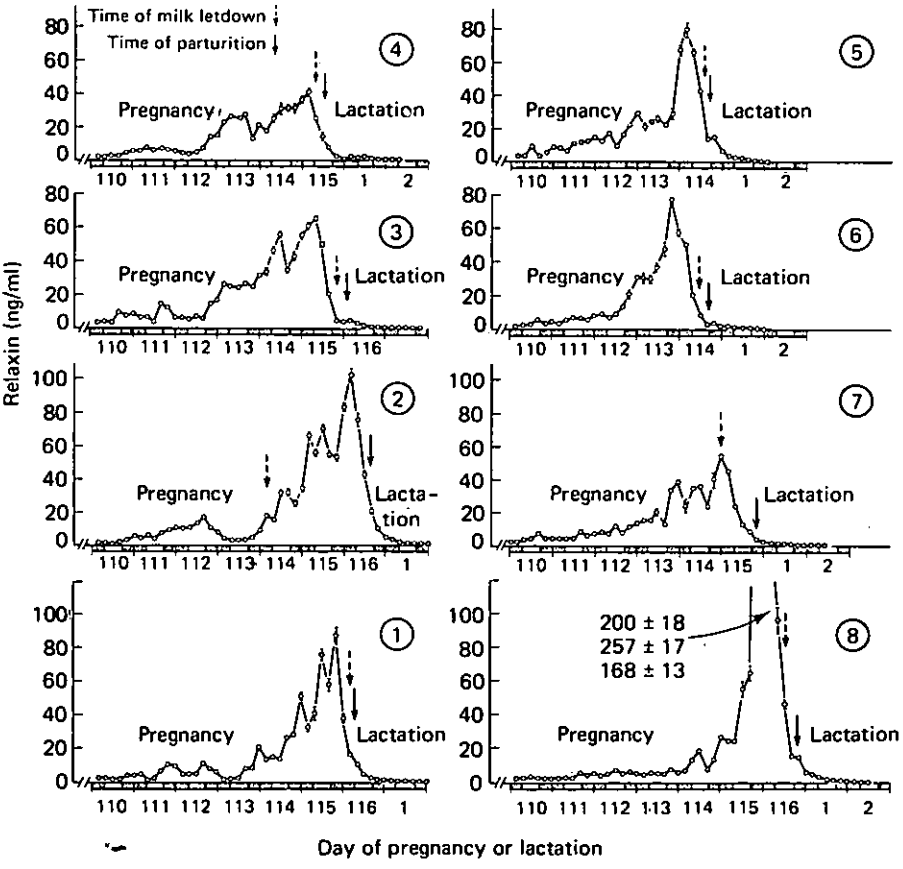

Figure 17.6 Relaxin concentrations (±S.E.M.) in peripheral plasma obtained from eight pigs at 4-hour intervals from 04.00 hours on day 110 until approximately 37 hours following parturition. The alternating dark and light bars along the abscissas indicate the periods of darkness and artificial lighting, respectively. From Sherwood *et al.* (1981)

(*Figure 17.7*) and continues following birth of the piglets (*Figure 17.8*). By 37 hours after parturition, relaxin levels in the plasma are less than 0.5 ng/ml. The range of relaxin concentrations obtained in the plasma during delivery (*Figure 17.7*) is in general agreement with that reported by Afele *et al.* (1979); however, unlike those workers this work (Sherwood *et al.*, 1981) which included blood samplings taken as frequently as 2-minute intervals, failed to show marked fluctuations in relaxin levels during parturition or during suckling. Since the clearance $t_{½}$ for relaxin in the pig is approximately 60 minutes (Sherwood, 1982b), it seems likely that marked fluctuations in serum relaxin levels during parturition or suckling would have been detected in the study of Sherwood *et al.* (1982) had they occurred.

One must be careful in the interpretation of polypeptide hormone levels obtained by radioimmunoassay since the radioimmunoassay does not necessarily measure biologically active molecules. It seems likely, however, that the immunoactive 'substance' measured with our porcine relaxin

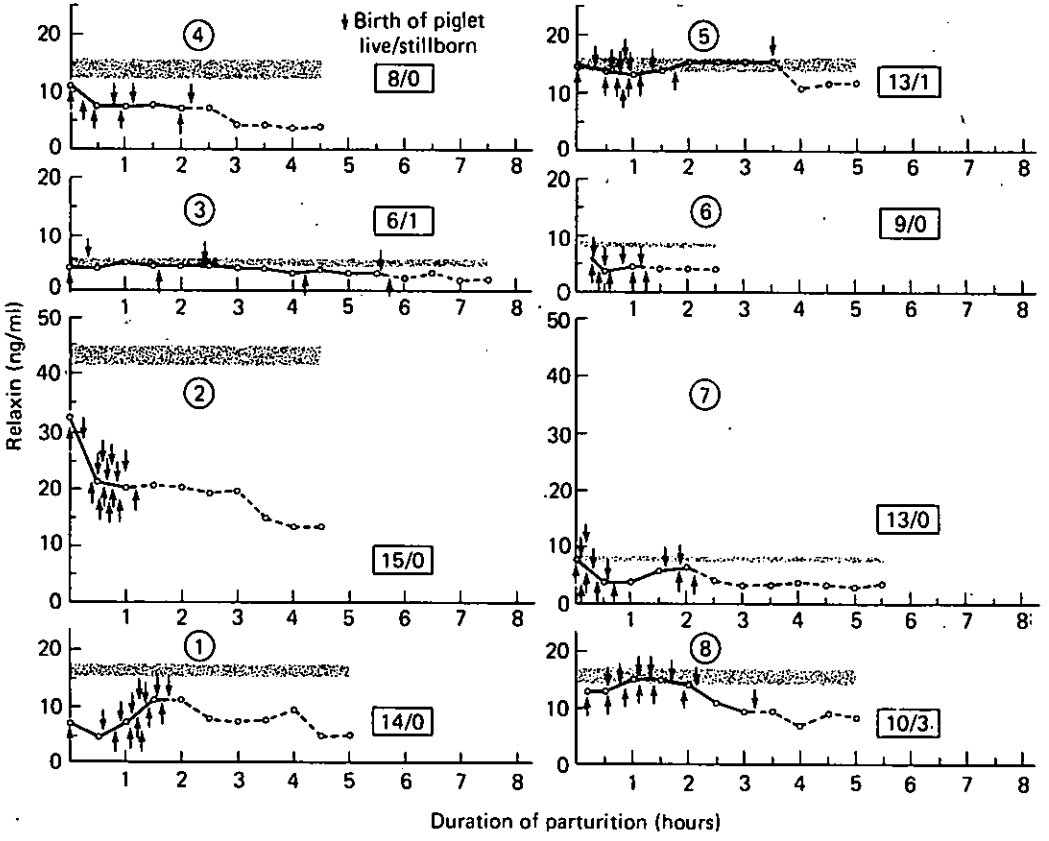

Figure 17.7 Relaxin concentrations in peripheral plasma obtained from eight pigs at 30 minute intervals throughout parturition. The horizontal shaded bars indicate the relaxin level (±S.E.M.) in the last plasma sample obtained before parturition. These bars have been extended to facilitate comparison with relaxin levels obtained during parturition. The solid lines indicate the duration of delivery of the piglets and the intermittent extension of each line indicates the duration of the delivery of the placentae. Similarly, plasma levels obtained in

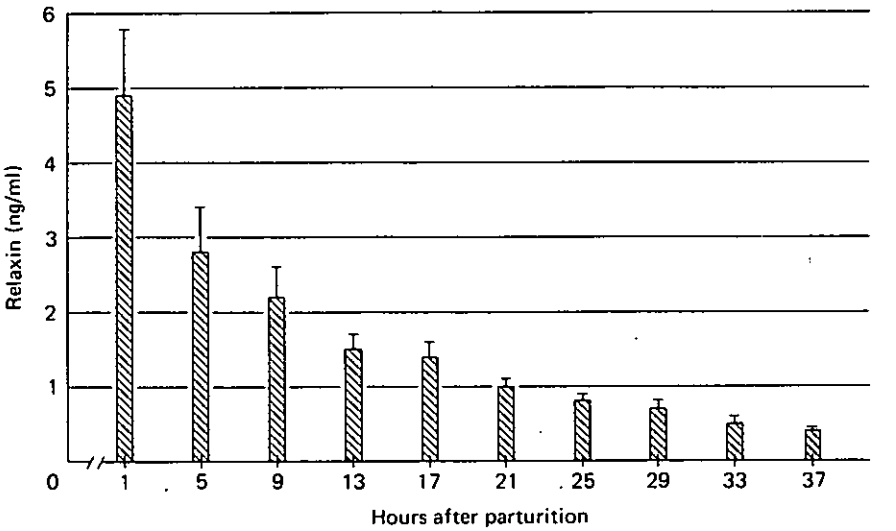

Figure 17.8 Relaxin concentrations in peripheral plasma obtained at 4 hour intervals from 1 hour to 37 hours following parturition. This figure contains the means (±S.E.M.) of the eight animals shown in *Figures 17.6* and *17.7*. From Sherwood *et al.* (1981)

radioimmunoassay is, at least in part, biologically active relaxin since the pre-partum occurrence of high blood levels of relaxin immunoactivity (*Figure 17.6*) and relaxin bioactivity (Belt *et al.*, 1971) coincide.

Association of the pre-partum release of relaxin from the corpora lutea with luteal regression

In the pregnant pig the corpora lutea are the primary source of both progesterone and relaxin (Belt *et al.*, 1971; Anderson *et al.*, 1973; Corteel, Lemon and Dubois, 1977; Larkin, Fields and Oliver, 1977; Sherwood *et al.*, 1977a; Dubois and Dacheux, 1978; Kendall, Plopper and Bryant-Greenwood, 1978; Arakaki, Kleinfeld and Bryant-Greenwood, 1980). Since the corpora lutea are undergoing functional regression, as judged by a rapid fall in progesterone levels during the two days which precede parturition (Molokwu and Wagner, 1973; Killian, Garverick and Day, 1973; Baldwin and Stabenfeldt, 1975; Ash and Heap, 1975), it seemed evident that the simultaneous pre-partum elevation in peripheral plasma relaxin levels might also be associated with luteal regression. This possibility has been explored. Pregnant pigs were given surgical or pharmacological treatments which might influence luteal function during late pregnancy in order to determine whether these treatments also influenced pre-partum levels of relaxin in the peripheral blood.

INFLUENCE OF ALTERED UTERO-OVARIAN RELATIONSHIP

There is evidence that the factor(s) which bring about luteolysis during late pregnancy can be carried in the systemic circulation. Martin, BeVier and Dziuk (1978) demonstrated that luteolysis and parturition occurred at the

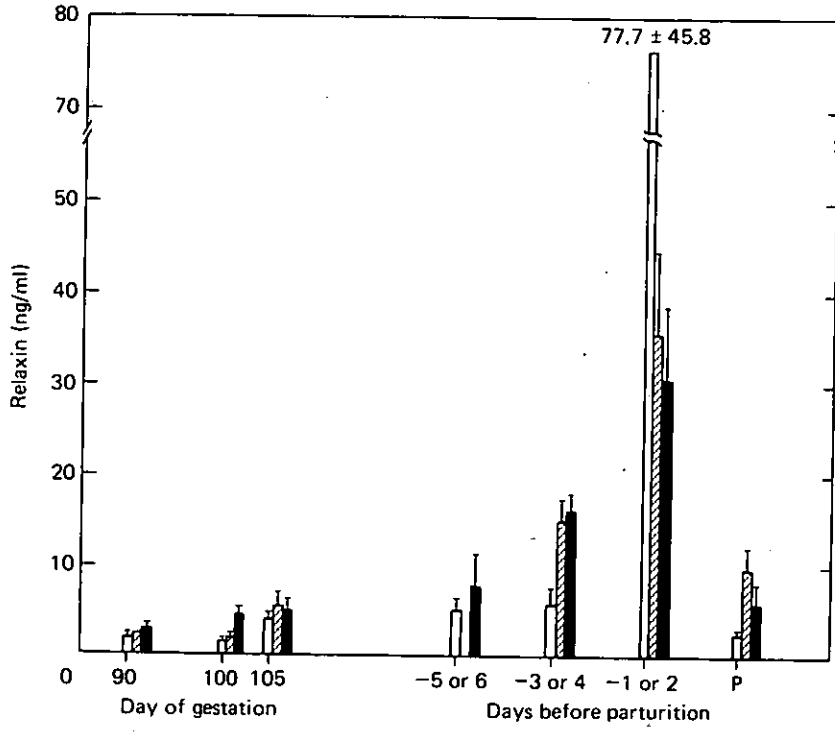

Figure 17.9 Mean relaxin concentrations (±S.E.M.) in peripheral plasma obtained during late pregnancy in pigs with altered utero-ovarian connections. □—Group I comprising seven gilts in which the ovaries were transplanted to the adjacent uterine wall. Blood was collected at two day intervals. ▨—Group II comprising three gilts in which the ovaries were transplanted to the exterior abdominal wall. ■—Group III comprising four gilts in which one uterine horn and its contralateral ovary were removed. P = day of parturition. From Sherwood *et al.* (1977b)

expected time in pigs whose normal utero-ovarian relationship was altered so that there were no direct utero-ovarian connections. Likewise, the pre-partum elevation of relaxin levels in the blood of these pigs occurred at the expected time and concomitant with luteolysis (*Figure 17.9*). Therefore, it appears that the factor(s) which brings about the pre-partum elevation of relaxin levels, as well as that which brings about luteolysis, can be carried in the systemic circulation.

INFLUENCE OF PROSTAGLANDINS

The nature of the factors which bring about luteolysis and the pre-partum elevation in relaxin levels are not known with certainty. However, there is evidence that prostaglandins may be involved with both phenomena (Diehl *et al.*, 1974; Sherwood *et al.*, 1976; 1979; Nara and First, 1981). *Figure 17.10* shows that the infusion of sufficient $PGF_{2\alpha}$ on day 110 to induce parturition on day 111 brought about a drop in progesterone concentrations and a surge in relaxin levels in peripheral plasma. Additionally, daily

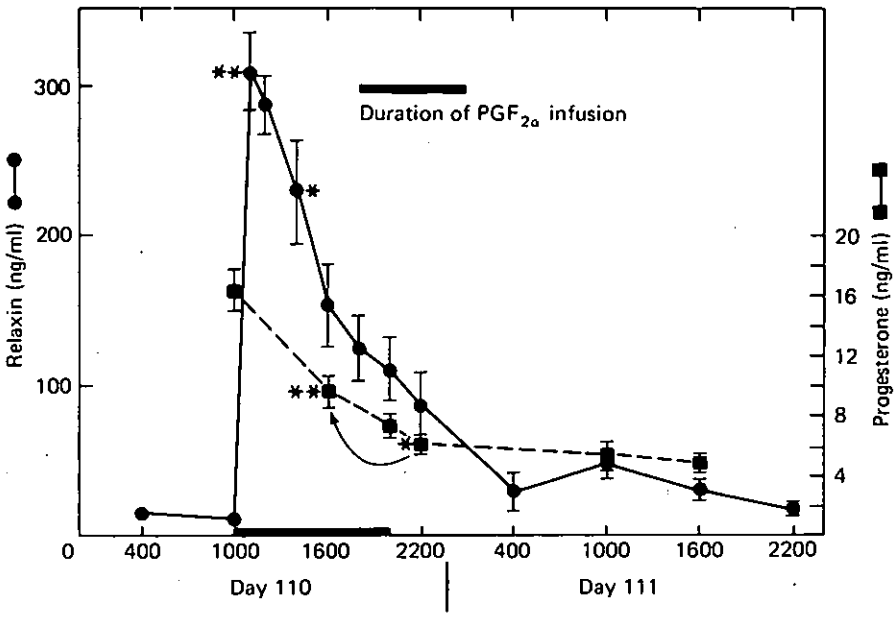

Figure 17.10 Mean relaxin and progesterone levels (± S.E.M.) in peripheral plasma obtained from five pigs which were infused with prostaglandin $F_{2\alpha}$ at a rate of 0.5 mg/hr over a 10 hour period on day 110. Asterisks denote those mean hormone concentrations which differ significantly (* $P<0.05$; ** $P<0.01$) from those which immediately precede them or those indicated with an arrow. From Sherwood *et al.* (1979)

injection of the prostaglandin synthesis inhibitor indomethacin from day 109 to day 116 delayed both the elevation of relaxin levels and parturition, which normally occur between day 112 and 116, until 2–4 days after the termination of indomethacin administration (*Figure 17.11*). Likewise, the decline in progesterone concentrations in indomethacin-treated pigs was delayed and was concurrent with the surge in relaxin levels which occurred on approximately day 119 (*Figure 17.12*). It is not known whether luteolysis and the pre-partum release of relaxin into the blood are initiated by common or separate prostaglandin-mediated mechanisms.

INFLUENCE OF THE FOETUS

There is evidence that the foetal pituitary–adrenal–placental system controls the initiation of parturition in the pig (First and Bosc, 1979). Destruction of the foetal pituitary (Bosc, du Mesnil du Buisson and Locatelli, 1974) or foetal decapitation *in utero* (Stryker and Dziuk, 1975; Coggins and First, 1977) prolongs pregnancy. The ablation of the foetal pituitary apparently prolongs pregnancy by preventing the rapid and sustained luteolysis which normally occurs on approximately days 113–115 (Fevre, Terqui and Bosc, 1975; Coggins and First, 1977). Ablation of the foetal pituitary by foetal decapitation (Sherwood, Hagen, Dial and Dziuk,

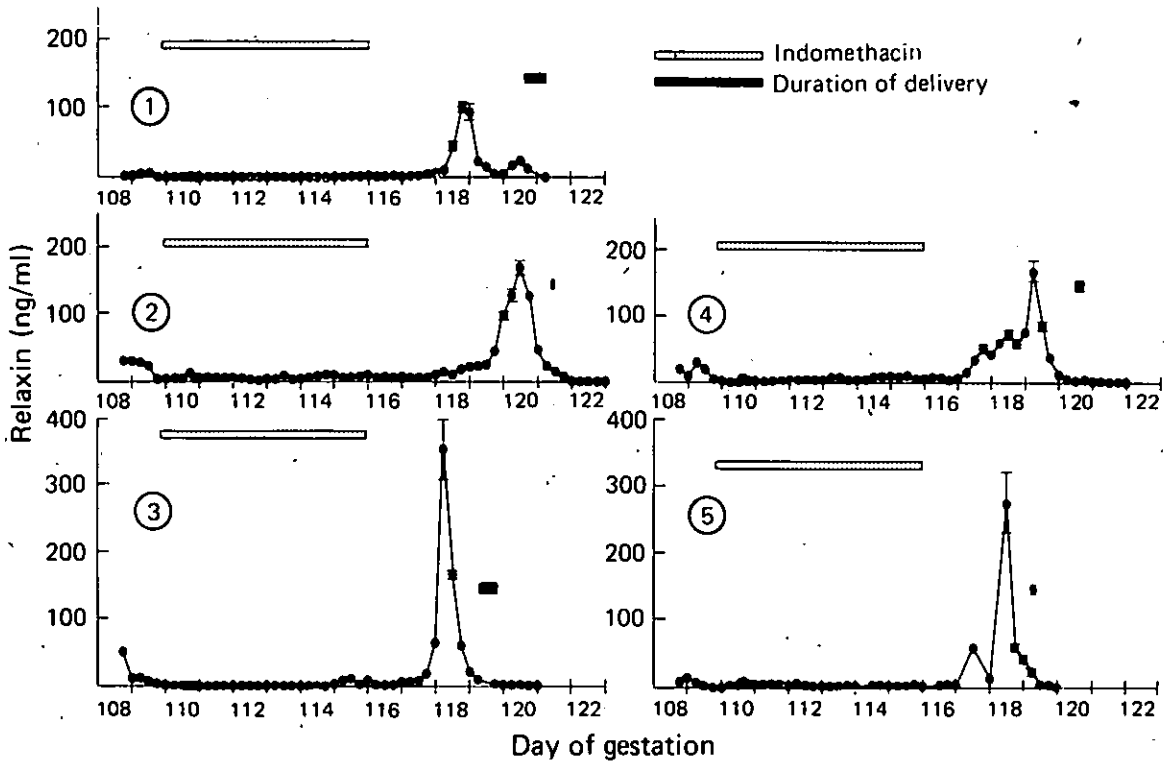

Figure 17.11 Relaxin concentrations (± S.E.M.) in peripheral plasma obtained at 6 hour intervals from 16.00 hours on day 108 until 24 hours after parturition in five pigs injected i.m. twice each day with indomethacin at a dose of 4 mg/kg from day 109 to day 116. From Sherwood *et al.* (1979)

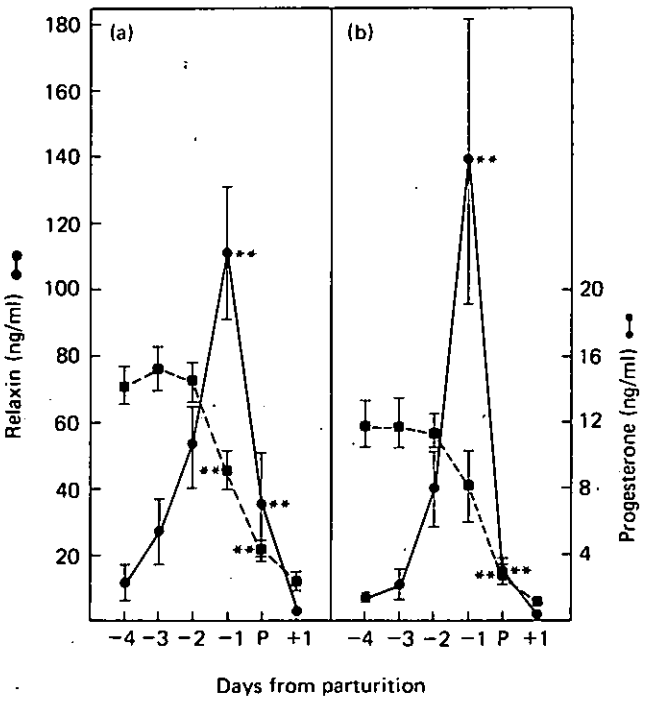

Figure 17.12 Mean relaxin and progesterone concentrations (±S.E.M.) in peripheral plasma obtained from control (a) and indomethacin-treated (b) pregnant pigs. The times of the initiation of delivery for control and the indomethacin groups were 114.9±0.5 (S.E.M.) and 120.1±0.4 (S.E.M.) days, respectively. Asterisks denote those mean hormone concentrations which differ significantly (**$P<0.01$) from those which immediately precede them. P = day of parturition. From Sherwood *et al.* (1979)

unpublished) or foetal hypophysectomy (Kendall *et al.*, 1980) also influences the pre-partum release of relaxin. *Figure 17.13(b)* shows that gilts which contained decapitated foetuses and failed to deliver by day 117 had elevated levels of relaxin in the peripheral plasma on one or more days near the time of normal parturition. However, unlike the gilts which contained intact foetuses (*Figure 17.13(a)*), the peaks of relaxin occurred randomly and were not related to any particular day of gestation or interval before parturition. Therefore, it appears that the foetus may play a role in the control of both luteolysis and the release of relaxin which normally occur during the two days which precede parturition.

The mechanisms which bring about luteolysis in the pregnant pig are not well understood. The apparent association of the pre-partum elevation in relaxin levels with luteolysis may provide a specific indicator for the study of luteal regression. It may be that the multiple and generally progressively greater surges in relaxin levels detected during the last 2–4 days of pregnancy (*Figure 17.6*) are a manifestation of an underlying intermittent and increasingly effective luteolytic mechanism which ultimately brings about the rapid decline in plasma progesterone levels which occurs during

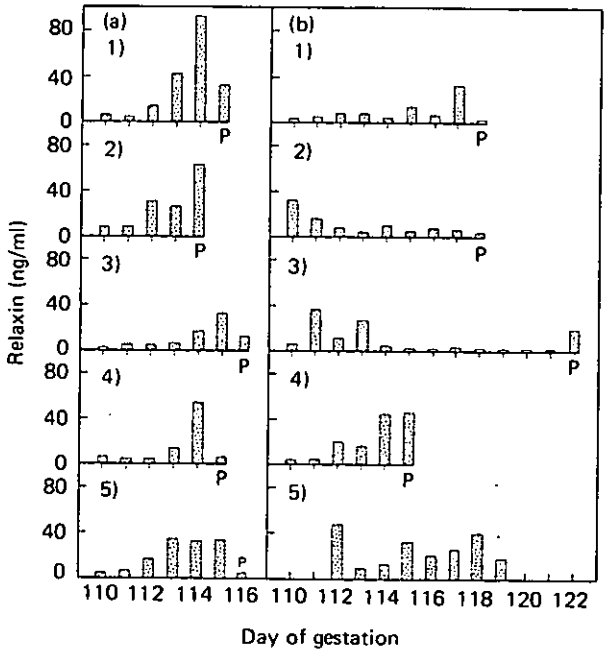

Figure 17.13 Relaxin concentrations in peripheral plasma obtained at daily intervals during late pregnancy from (a) control pigs and (b) pigs containing decapitated foetuses. P = day of parturition. With animal number 5 in treatment group (b) the foetuses were removed surgically by hysterotomy on day 119. From Sherwood, Hagen, Dial and Dziuk (unpublished)

this time. The author is not aware that an intermittent luteolytic mechanism has been previously identified or suggested for the pig during late pregnancy.

Physiological effects of relaxin

The observations that relaxin levels increase in the corpora lutea during pregnancy (Hisaw and Zarrow, 1948; Belt *et al.*, 1971; Anderson *et al.*, 1973) and that relaxin levels increase in the blood during the last 10–14 days of pregnancy (Sherwood, Rosentreter and Birkhimer, 1975) indicate that relaxin may have important physiological functions during pregnancy and parturition in the pig. However, the physiological effects of relaxin in the pregnant pig have not been extensively studied and are not well understood. Accordingly, to a large degree, this section contains conclusions and hypotheses which are based on limited studies, which in most cases employed impure porcine relaxin preparations and species other than the pig, or inferences drawn from indirect observations. Nevertheless, consideration of those studies which have been done seems important since additional research concerning the physiological effects of relaxin in the pig are needed, and the insights and inferences drawn from past studies may contribute toward well-designed and productive future experiments.

EFFECT OF RELAXIN ON PARTURITION

There is evidence that relaxin promotes a high rate of livebirths and may do so by contributing to a short duration of delivery of the piglets. Kertiles and Anderson (1979) reported that daily intramuscular injections of partially purified porcine relaxin beginning on days 105 or 107 and before surgical enucleation of corpora lutea (lutectomy) on day 110 significantly reduced the duration of delivery of all neonates in the litter compared with lutectomized controls. In a recent experiment we abolished the elevated levels of relaxin normally experienced during late pregnancy by bilaterally ovariectomizing pigs on day 105 and then maintained pregnancy by progesterone administration (Nara *et al.*, submitted for publication). When

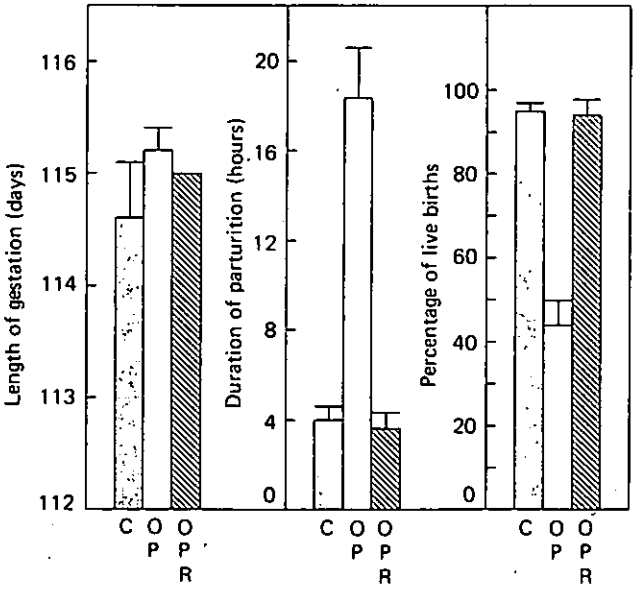

Figure 17.14 The effects of highly purified relaxin on parturition. The five gilts in control group C were sham ovariectomized on day 105 and given intramuscular injections of (1) 4 ml of corn oil at 12 hour intervals from 18.00 hours on day 105 to 18.00 hours on day 112, and (2) 1 ml of 0.9% saline at 6 hour intervals from 12.00 hours on day 105 until parturition was completed. The five gilts in treatment group OP were bilaterally ovariectomized and were injected with (1) 100 mg progesterone in 4 ml of corn oil and with (2) 1 ml of 0.9% saline according to the injection regimen used with treatment C for corn oil and saline alone. The five gilts in treatment group OPR were ovariectomized and treated with progesterone as those in treatment group OP, but were given intramuscular injections of 1 mg of highly purified porcine relaxin in 1 ml of 0.9% saline instead of the injections of saline alone. From Nara *et al.* (submitted for publication)

parturition was induced by progesterone withdrawal on day 112, the duration of parturition was prolonged, and the incidence of piglets born alive was much lower than controls (*Figure 17.14*). Replacement therapy with progesterone plus physiological levels of highly purified porcine relaxin restored the duration of parturition and the incidence of livebirths to values similar to those of controls. It seems likely that the capacity of

relaxin to promote livebirths is associated with its effect on the duration of parturition, since a high incidence of stillbirths has been reported to be associated with a prolonged duration of farrowing (Friend, Cunningham and Nicholson, 1962; Randall, 1972).

EFFECT OF RELAXIN ON THE UTERINE CERVIX

The mechanism(s) whereby relaxin promotes a short duration of delivery and high incidence of livebirths in the pig is not well understood. There is evidence that relaxin may do so, at least in part, through direct effects on the uterine cervix which will hereafter be referred to as the cervix. McMurtry, Kwok and Bryant-Greenwood (1978) reported that ^{125}I-labelled porcine relaxin bound with the characteristics of a hormone–receptor interaction to a homogenate of guinea pig cervix. Additionally, it has recently been demonstrated that the levels of cAMP increased in rat (Cheah and Sherwood, 1980; Sanborn *et al.*, 1980) and pig (Judson, Pay and Bhoola, 1980) cervical tissue following *in vitro* incubation with porcine relaxin.

Several lines of evidence indicate that relaxin may be associated with changes in the physical properties of the cervix which occur during pregnancy. The literature describes these cervical changes in a variety of ways including 'ripening', 'softening', 'increased compliance', 'increased dilatation', 'increased extensibility', and 'increased distensibility'. In the interest of brevity, the cervical changes characteristic of pregnancy will generally be referred to as increased distensibility. Several ultrastructural and biochemical changes have been reported which may influence the changes in the physical properties of the cervix which occur during pregnancy. These include increased cervical water content, increased secretion of proteolytic enzymes by cervical fibroblasts, loosening of the collagenous fibre network, and alterations in the extracellular glycosaminoglycans composition (Steinetz, O'Byrne and Kroc, 1980; Golichowski, 1980; Veis, 1980). The biochemical mechanisms associated with the cervical changes which occur during pregnancy and the influence of hormones on these mechanisms remain poorly understood and will not be further discussed in this chapter.

Relaxin appears to be associated with the increased distensibility of the cervix which occurs during pregnancy in rodents. In the rat increased cervical distensibility occurs after day 11 or 12 (Uyldert and DeVaal, 1947; Harkness and Harkness, 1959; Kroc, Steinetz and Beach, 1959; Zarrow and Yochim, 1961; Hollingsworth, Isherwood and Foster, 1979) and coincides with the occurrence of elevated levels of relaxin in the peripheral blood (O'Byrne and Steinetz, 1976; Sherwood *et al.*, 1980). Similar close correlations between increased cervical distensibility and serum levels of relaxin immunoactivity have been described for the pregnant mouse (Steinetz, O'Byrne and Kroc, 1980) and hamster (O'Byrne *et al.*, 1976). When rats were ovariectomized on days 12, 15 or 16 and pregnancy was maintained by injections of progesterone and oestradiol, the marked increase in cervical distensibility normally observed during late pregnancy did not occur (Kroc, Steinetz and Beach, 1959; Steinetz, Beach and Kroc,

1959; Zarrow and Yochim, 1961; Hollingsworth, Isherwood and Foster, 1979). However, when porcine relaxin was given to similarly treated rats, there was a marked increase in cervical distensibility (Kroc, Steinetz and Beach, 1959; Steinetz, Beach and Kroc, 1959; Zarrow and Yochim, 1961; Hollingsworth, Isherwood and Foster, 1979).

Relaxin may also influence cervical changes in the pregnant human being. MacLennan *et al.* (1980) recently reported that the placement of a viscous gel containing 2 mg of highly purified porcine relaxin into the posterior vaginal fornix of women the evening before surgical induction of labour resulted in improved cervical scores. Similar cervical changes have also been observed in non-pregnant ovariectomized rats, mice, monkeys and heifers following treatment with relaxin and oestradiol or relaxin,

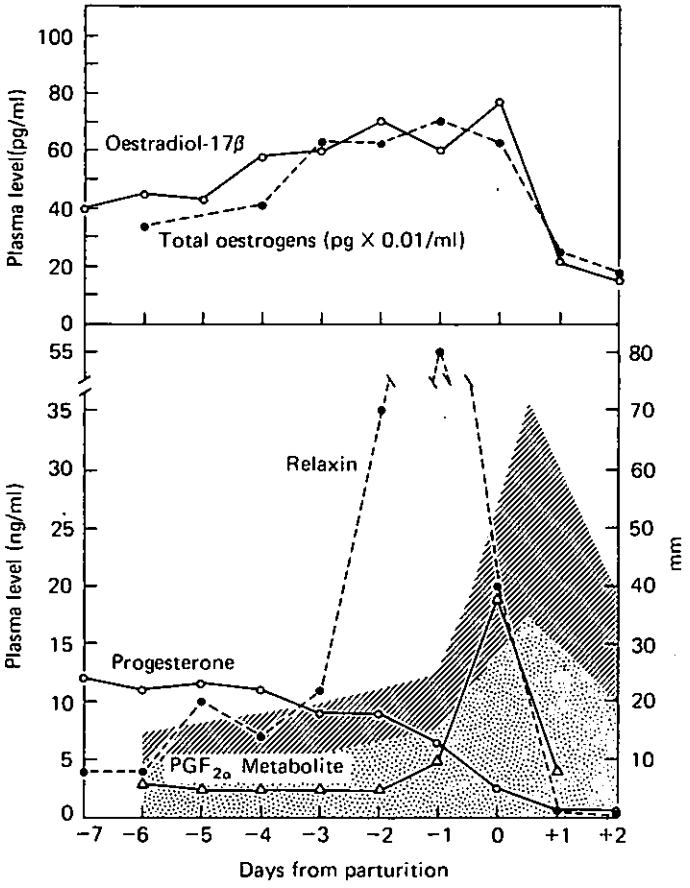

Figure 17.15 ▨—Cervical dilatation (mm), ▩—cervical diameter (mm), and peripheral plasma levels of relaxin, progesterone, $PGF_{2\alpha}$ metabolite, oestradiol-17β, and total oestrogens. Dilatation of the cervix of four gilts was determined by inserting aluminum rods of different diameters into the cervix (from Kertiles and Anderson, 1979). Data from the following reports were used to prepare this figure: Oestradiol-17β (Molokwu and Wagner, 1973), total oestrogens (Baldwin and Stabenfeldt, 1975), progesterone (Molokwu and Wagner, 1973), $PGF_{2\alpha}$ metabolite (Nara and First, 1981), relaxin (Sherwood *et al.*, 1975b)

oestradiol and progesterone, but not following treatment with these steroids alone (Graham and Dracy, 1953; Zarrow, Sikes and Neher, 1954; Kroc, Steinetz and Beach, 1959; Steinetz, Beach and Kroc, 1959; Cullen and Harkness, 1960; Hall, 1960; Zarrow and Yochim, 1961; Leppi, 1964; Hisaw and Hisaw, 1964; Kennedy, 1974; Fields and Larkin, 1980).

There have been few studies concerning the effects of relaxin on the cervix in the pig. Zarrow *et al.* (1956) reported that three daily intramuscular injections of partially purified porcine relaxin (approximately 5 mg relaxin/day) for four days to non-pregnant ovariectomized sows which had been pretreated for seven days with 5 mg of diethylstilboestrol caused a dilatation of the cervix which was accompanied by an increase in cervical water content and depolymerization of cervical glycoproteins. A recent study supports the possibility that relaxin may promote increased distensibility of the cervix during late pregnancy in the pig. Kertiles and Anderson (1979) demonstrated that the cervical diameter in pregnant pigs did not increase until late pregnancy when relaxin levels in the blood are elevated (*Figure 17.15*). Additionally, these workers reported that daily intramuscular injections of a small amount of partially purified porcine relaxin (approximately 250 μg relaxin) beginning on days 105 or 107 induced premature cervical dilatation in pigs which were lutectomized on day 110.

EFFECT OF OTHER HORMONES ON THE UTERINE CERVIX

Studies which have largely been conducted with species other than the pig indicate that the cervical modifications which occur near parturition may be influenced not only by relaxin but also directly or indirectly by other hormones. *Figure 17.15* shows pre-partum blood levels of relaxin, prostaglandin $F_{2\alpha}$ metabolite, progesterone, and oestrogen—three hormones and the metabolite of a hormone which may influence cervical function at parturition in the pig.

Prostaglandins

Evidence acquired from studies with several species indicates that the prostaglandins and perhaps prostacyclin may influence cervical distensibility during late pregnancy and at parturition. (For reviews see McInnes, Naftolin and van der Rest, 1980; Fitzpatrick and Liggins, 1980.) Hollingsworth, Gallimore and Isherwood (1980) reported that $PGF_{2\alpha}$ and PGE_2 caused increased cervical distensibility in pregnant rats. These workers suggested that $PGF_{2\alpha}$ may act on the ovaries to decrease progesterone secretion and perhaps release relaxin, whereas PGE_2 may act directly on cervical tissue. It has also been suggexted that prostaglandins may mediate the effect of relaxin on the rat cervix. Kennedy (1976) administered oestrogen in order to constrict the cervix of ovariectomized non-pregnant rats and then found that the effectiveness with which relaxin released accumulated intraluminal secretions was blocked by the administration of indomethacin. There is evidence that prostaglandins may have direct effects on the cervix in human beings. Intravaginal administration of PGE_2

or $PGF_{2\alpha}$ (Dingfelder *et al.*, 1975; Mackenzie and Embrey, 1977) or *in vitro* administration of PGE_2 (Najak, Hillier and Karim, 1970; Conrad and Ueland, 1976) enhanced ripening or elasticity of human cervical tissue. Likewise, continuous infusion or gel suspension of PGE_2 into the extra-amniotic space has been used for the induction of cervical ripening in women (Calder, 1980). Prostaglandins and/or prostacyclin may promote cervical distensibility in sheep. Infusion of PGE_2 or $PGF_{2\alpha}$ directly into the cervical lumen of pregnant sheep during late pregnancy was reported to produce cervical softening (Fitzpatrick, 1977). More recently, however, Fitzpatrick and Liggins (1980) reported that it may be prostacyclin and not prostaglandins which normally promotes cervical distensibility in pregnant sheep; when parturition was induced by means of a continuous five-day infusion of ACTH into the foetus, the levels of the stable prostacyclin breakdown product 6-keto-$PGF_{1\alpha}$ increased in uterine veins approximately three days before elevated levels of PGF were detected. There are also data indicating that $PGF_{2\alpha}$ may not be associated with cervical distensibility which occurs during late pregnancy in goats. Increased compliance of the cervix occurred several hours before increased levels of PGF were detectable in the blood of goats in which parturition was induced with a synthetic prostaglandin (Fitzpatrick and Liggins, 1980). Neither the effects of prostaglandins nor prostacyclin on the cervix of the pregnant pig have been reported.

Progesterone

The effects of progesterone on the cervix are not clearly understood. Studies with ovariectomized rats and mice indicate that the administration of progesterone alone has little effect on the distensibility of the cervix (Kroc, Steinetz and Beach, 1959; Cullen and Harkness, 1960; Zarrow and Yochim, 1964; Leppi, 1964). However, progesterone has been reported to bring about some augmentation of the stimulating effects of relaxin on cervical distensibility in ovariectomized rats and mice which were primed with oestrogen and treated with relaxin and progesterone (Kroc, Steinetz and Beach, 1959; Cullen and Harkness, 1960; Leppi, 1964). Progesterone does not appear to inhibit the cervical distensibility which occurs at parturition in sheep. Stys *et al.* (1980) demonstrated that the daily administration of 200 mg of progesterone to ewes in which parturition was induced by the infusion of dexamethasone into the foetus failed to inhibit an increase in cervical compliance. The effects of progesterone on the cervix during late pregnancy in the pig have not been extensively studied. The limited data available indicate that progesterone does not promote cervical distensibility. The marked increase in cervical distensibility which occurs during late pregnancy coincides with a decline in progesterone levels in the peripheral plasma (*Figure 17.15*). In pregnant pigs in which parturition was delayed for 3–7 days by the administration of exogenous progestin, there was a marked decline in livebirths (Nellor *et al.*, 1975; Sherwood *et al.*, 1978) which may have been, at least in part, attributable to incomplete cervical dilatation (Nellor *et al.*, 1975). The apparent failure of normal cervical dilatation at parturition in progestin-treated pigs (Nellor

et al., 1975) may not be attributable to a relaxin deficiency, since elevated levels of relaxin occurred at the expected time (approximately day 113) in similarly treated animals (Sherwood *et al.*, 1978). Results obtained with non-pregnant pigs are consistent with the view that progesterone does not increase the distensibility of the cervix. The daily injection of progesterone did not promote an increase in the distensibility of the cervix in sows in oestrus (Smith and Nalbandov, 1958) or in ovariectomized sows regardless of whether the animals were treated with progesterone alone (Zarrow *et al.*, 1956) or with progesterone and oestrogen (Smith and Nalbandov, 1958; Zarrow *et al.*, 1956).

Oestrogens

Oestrogens also influence cervical distensibility. In rodents, oestrogens stimulate an increase in cervical weight (Kroc, Steinetz and Beach, 1959; Cullen and Harkness, 1960; Zarrow and Yochim, 1961; Leppi, 1964) and apparently bring about changes which enable relaxin to increase cervical distensibility. When non-pregnant ovariectomized mice (Leppi, 1964) or rats (Kroc, Steinetz and Beach, 1959; Cullen and Harkness, 1960) were injected with either oestrogen or porcine relaxin alone, there was little effect on cervical distensibility, whereas when relaxin was administered to similar animals which had been pretreated with oestrogen, there was a marked increase in cervical distensibility (Leppi, 1964; Kroc, Steinetz and Beach, 1959; Cullen and Harkness, 1960; Kennedy, 1974). In sheep the subcutaneous injection of 20 mg of diethylstilboestrol on approximately day 130 of pregnancy promoted cervical compliance (Stys *et al.*, 1980), and a single subcutaneous injection of 5 mg of oestradiol benzoate between days 127 and 128 of pregnancy increased cervical weight and dilatation (Fitzpatrick and Liggins, 1980). Oestrogens also promote cervical ripening in pregnant women. Systemic administration of oestradiol-17β (Pinto *et al.*, 1964) or dehydroepiandrosterone sulphate (Mochizuki and Tojo, 1980) during late pregnancy increased ripening of the cervix. Oestrogen may act directly on the cervix, since the extra-amniotic placement of oestradiol in a viscous gel promoted increased cervical ripening (Gordon and Calder, 1977).

The above observations raise fundamental and important questions concerning the role of relaxin on the pig cervix. Does relaxin play an important role in promoting the increased distensibility of the cervix which occurs during late pregnancy? If so, what are the effects of relaxin on the morphology and biochemistry of the pig cervix? Do these effects involve interactions with other hormones such as prostaglandins, progesterone and oestrogen and, if so, what is the nature of these interactions? Intensified efforts to answer these questions seem worthwhile.

EFFECT OF RELAXIN ON UTERINE CONTRACTILE ACTIVITY

It seems likely that the uterine horns are also a target tissue for relaxin. Intact uterine horns (Cheah and Sherwood, 1980) or slices of uterine horns

(Mercado-Simmen, Bryant-Greenwood and Greenwood, 1980) obtained from rats bound ^{125}I-labelled porcine relaxin with characteristics of a hormone–receptor interaction. Relaxin may exert its effects on the uterus through cAMP, since levels of cAMP increased in pieces of rat (Cheah and Sherwood, 1980; Sanborn *et al.*, 1980; Judson, Pay and Bhoola, 1980) and pig (Judson, Pay and Bhoola, 1980) uterine horn following incubation with porcine relaxin.

The effects of relaxin on the uterine horns are not well understood. Relaxin may influence uterine contractile activity during pregnancy. Both *in vivo* and *in vitro* studies have demonstrated that partially purified preparations of porcine relaxin reduce the frequency of uterine contractile activity in the guinea pig (Krantz, Bryant and Carr, 1950; Porter, 1971; 1972), rat (Sawyer, Frieden and Martin, 1953; Porter, Downing and

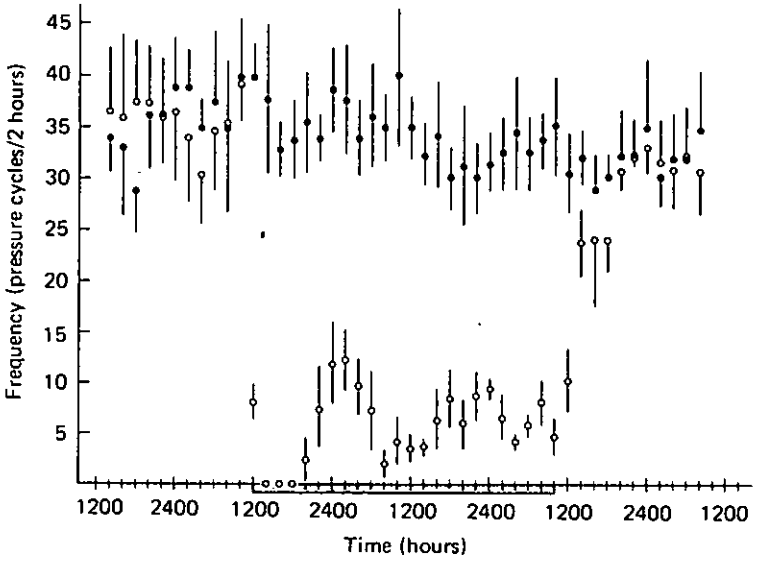

Figure 17.16 The effect of infusion of highly purified porcine relaxin (20 μg/hour) on the frequency of contractions of the uterus in conscious and unrestrained oestrogen-treated ovariectomized rats. The frequency of contractions (±S.E.M.) in four relaxin-treated rats (○) and three control rats (●) are shown. The period of infusion of the relaxin is shown by the stippled bar. From Cheah and Sherwood (1981)

Bradshaw, 1979; Chamley, Bagoyo and Bryant-Greenwood, 1977), mouse (Wiqvist, 1959) and hamster (Khaligh, 1968). The inhibitory effect of relaxin on rat uterine myometrial activity has recently been confirmed by studies which employed highly purified porcine relaxin (*Figure 17.16*; Sanborn *et al.*, 1980). Porter and his colleagues provided compelling evidence that relaxin may restrain uterine contractile activity during late pregnancy. They demonstrated with cross-circulation techniques that a myometrial inhibitor(s) with a more rapid onset of activity than oestrogen or progesterone is present in the blood of the guinea pig (Porter, 1972), rabbit (Porter, 1974), and rat (Porter and Downing, 1978) during late pregnancy when the levels of relaxin in the blood of these species are

elevated (Zarrow and Rosenberg, 1953; O'Byrne and Steinetz, 1976; Sherwood *et al.*, 1980). Additionally, it has been reported that the frequency of uterine contractions diminishes during the second half of pregnancy in the rat (Fuchs, 1978) when relaxin immunoactivity levels in the blood are elevated. Based largely on the results of the studies described above, it has been hypothesized that relaxin may provide a mechanism which protects the foetuses and placentas during the period of progesterone withdrawal just before parturition in rabbits and rats by restraining spontaneous uterine contractile activity (Porter, 1974; 1979a). Such a protective mechanism may be important during late pregnancy. It is known that in the rat as the uterus progressively comes under greater oestrogen domination during late pregnancy (Yoshinaga, Hawkins and Stocker, 1969), its capacity for strong highly coordinated contractions increases (Fuchs, 1978). In the presence of elevated blood levels of oestrogen, there is an increase in electrical conductivity (Melton and Saldivar, 1964), uterine prostaglandin production (Harney, Sneddon and Williams, 1974; Carminati, Luzzani and Lerner, 1976), uterine oxytocin receptor concentrations (Alexandrova and Soloff, 1980a; 1980b) and responsiveness of the uterus to oxytocin (Fuchs and Poblete, 1970; Fuchs, 1978). Relaxin may inhibit uterine contractions until overriden by stimulating agents such as oxytocin and prostaglandins or other mechanisms which bring about the onset of strong, highly coordinated uterine contractions and the delivery of the foetuses.

At this time there is no direct evidence that relaxin inhibits uterine contractions in the pig. However, the results of a recent study of the myometrial electrical activity of the miniature pig during the last 21 days of pregnancy are consistent with the hypothesis that relaxin may suppress uterine contractions during late pregnancy in the pig. Taverne *et al.* (1979) reported that as late as the period between 24 hours and 10 hours before expulsion of the first piglet, the electrical activity of the myometrium had not changed even though the peripheral blood levels of progesterone had fallen and those of oestrogen had increased. The evolution of regular and frequent phases of myometrial activity which occurred during the 9 hour period before parturition in these pigs coincided with increasing maternal plasma oxytocin concentrations. In agreement with Porter (1979a), these workers suggested that relaxin may act as a secondary myometrial inhibitor during the period of progesterone withdrawal.

The effects of relaxin on uterine contractile activity may be influenced by several hormones including progesterone, oestrogens, oxytocin, catecholamines, and prostaglandins whose concentrations change during late pregnancy. In order to gain a good understanding of the effect(s) of relaxin on uterine contractile activity in the pregnant pig, experiments are needed which recognize that these fluctuations in hormone concentrations are probably accompanied by changes in the responsiveness of the uterus to these hormones.

OTHER EFFECTS OF RELAXIN

There is evidence which has been acquired almost entirely from studies on species other than the pig which indicates that relaxin may have effects in

addition to those on cervical distensibility and uterine contractile activity. It has been reported that the administration of porcine relaxin to ovariectomized oestrogen-primed rats brings about a rapid increase in water content, dry weight, nitrogen content and glycogen content in the uterus (Steinetz *et al.*, 1957; Vasilenko, Frieden and Adams, 1980). Hall (1960) reported that most of the glycogen was found in the myometrium in mice. It is not known whether the effects of relaxin on glycogen levels in the rat uterus reflect a direct stimulation of glycogen synthesis or an indirect reduction in glycogen breakdown which occurs in response to an inhibition of uterine contractile activity.

There are considerable data which indicate that in some species, including the guinea pig and mouse, relaxin plays a role in bringing about modifications in the pubic symphysis which enable the pelvic girdle to be distended during parturition (for reviews see Schwabe *et al.*, 1978; Porter, 1979b).

Relaxin may also influence mammary gland growth and function. Treatment of ovariectomized rats with partially purified porcine relaxin together with oestrogen and progesterone reportedly increased growth, lobulation and DNA content of the mammary glands (Hamolsky and Sparrow, 1945; Smith, 1954; Harness and Anderson, 1975). The growth-promoting effects of relaxin on rat mammary glands has recently been confirmed with highly purified porcine relaxin (Wright and Anderson, 1981). Partially purified porcine relaxin has also been reported to depress milk yield in rats (Knox and Griffith, 1970) and goats (Cowie *et al.*, 1965). Recently, Kertiles and Anderson (1979) reported that lactation was reduced severely in intact and lutectomized pigs which were given partially purified porcine relaxin for several days during late pregnancy.

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