Direct venous–arterial transfer of ^{125}I -radiolabelled relaxin and tyrosine in the ovarian pedicle in sheep

W. Schramm, N. Einer-Jensen* and G. Schramm

Reproductive Endocrinology Program, The University of Michigan, Ann Arbor, MI 48109, U.S.A.

Summary. ^{125}I -labelled relaxin and tyrosine were infused into the ovarian vein to investigate transfer to branches of the ovarian artery at the ovarian pedicle in sheep. The ovarian arteries supply the ovary, the oviduct, and the tip of the uterine horn. An exchange of relaxin ($n = 24$) and tyrosine ($n = 18$) was observed in blood samples collected from all branches of ovarian arteries. This is expressed as a ratio of radioactivity > 1 between jugular venous blood plasma and arterial blood plasma. The average ratio ($\pm$ s.d.) over the total infusion period of 1 h was $1.42 (\pm 0.35)$ for relaxin and $1.69 (\pm 0.38)$ for tyrosine with maximal values up to 4.9 and 2.9, respectively. Of the total amount of substance infused (348 pmol/h), 0.22% of the relaxin and 1.19% of the tyrosine reached the adjacent arteries directly. From these investigations it is concluded that (a) molecules with a molecular weight of ~ 6000 can be transferred directly from veins to arteries at the ovarian pedicle, and (b) the efficiency of this exchange does not only depend on molecular size.

Introduction

Blood vessels of the reproductive tract in many mammals form a unique network with a large common contact area between veins and arteries (for review, see Ginther, 1976). For many substances, a local transfer from veins to arteries, thus 'short-circuiting' the peripheral blood circulation, has been shown (see McCracken, Schramm & Einer-Jensen, 1984). The focus of attention in most studies reported to date has been concentrated on steroids and prostaglandins. Radioactively labelled albumins (Coudert, Phillips, Faiman, Chernecki & Palmer, 1974; Einer-Jensen & McCracken, 1981) and methoxyinulin (Free & Jaffe, 1975) do not cross over from veins to arteries at structures with arterial rete contiguous to veins. In view of the recent reports that oxytocin is an ovarian hormone (for review, see Wathes, 1984), the question arises, can this and other potentially physiologically active polypeptides be locally transferred? However, with the exception of the polysaccharide methoxyinulin, to our knowledge no systematic studies have been published on the transfer of substances larger than steroids but smaller than albumins.

We have investigated the local transfer of oxytocin at the ovarian pedicle in sheep and found a transfer to branches of the ovarian arteries comparable in magnitude to the transfer of steroids (Schramm, Einer-Jensen, Schramm & McCracken, 1986). In this investigation, we have studied the transfer of relaxin and tyrosine at the ovarian pedicle. Relaxin was chosen as a model peptide of comparatively high molecular mass (~ 6000 ; Schwabe, McDonald & Steinetz, 1977; James, Niall, Kwok & Bryant-Greenwood, 1977) and the amino acid tyrosine was selected as a representative for one of the monomers constituting polypeptides.

Materials and Methods

Animals. Suffolk sheep (2–5 years of age) kept under natural Michigan conditions were used. The

* Present address: Department of Physiology, Odense University, Odense, Denmark.

experiments were carried out in the anoestrous season. Before surgery, food was withheld for 24 h but free access to water was allowed. On the day of the experiments, anti-bloating suspension Rumen-Eze (VET-A-MIX, Inc., Shenandoah, IO) and Turcapsol (Pitman-Moore, Inc., Washingtons Crossing, IL) were orally administered to retard ruminal fermentation.

Surgery. Experiments were carried out under general anaesthesia using sterile procedures. Anaesthesia was induced with i.v. administration of pentobarbitone sodium (12 mg/kg body weight). After insertion of an endotracheal tube, anaesthesia was maintained with halothane (0.5–2.0% volume: fluothane; Halocarbon Laboratories, Hackensack, NJ) in oxygen/nitrous oxide (1.0 l/min: 300 ml/min) in a semiclosed system with adsorption of carbon dioxide by soda lime. To prevent dehydration during surgery, lactated Ringer's solution (Abbott Laboratories, N. Chicago, IL) was administered at an infusion rate of 100 ml/h. The left jugular vein was cannulated for continuous collection of peripheral blood during the experiment at a rate of 300–500 μ l/min.

A midventral laparotomy was performed to gain access to the internal reproductive organs. The ovarian pedicle was exposed for cannulation with as little disturbance from its natural position as possible. The pedicle which allowed easiest access for dissection of blood vessels was chosen (6 left, 6 right). An ovarian vein was dissected free and cannulated 15 mm from the ovarian hilus. To achieve continuous collection of blood from ovarian arteries which were cannulated with tubing of only 0.28 mm i.d. (0.56 mm o.d.), this cannula was extended with 0.56 mm i.d. tubing attached at a distance of 50 mm from the cannulation point to reduce resistance to blood flow. Further details on surgical procedures are described elsewhere (Schramm *et al.*, 1986).

Relaxin. Porcine relaxin (NIH, essentially pure hormone; equivalent to 3000 units by the mouse pubic symphysis bioassay using the Werner-Lambert standard W1164 and pure NIH relaxin; see Porter, 1979) was reacted with methyl-*p*-hydroxybenzimidate (Wood, Wu & Gerhart, 1975) and iodinated with carrier free 125 I (NEN, Boston, MA) by means of chloramine-T (Hunter & Greenwood, 1962). Free iodide was separated from the radiolabelled protein by size exclusion chromatography on polyacrylamide gel (P-6, Bio-Rad, Richmond, CA). Radiolabelled relaxin (sp. act. 34 μ Ci/ μ g) was used for infusions within 2 days of preparation.

To investigate whether free 125 I or an iodinated fragment of relaxin is preferentially exchanged at the ovarian pedicle, three consecutively collected plasma samples from each vessel with the highest 125 I content were pooled and aliquants (0.5 ml) were purified by gel exclusion chromatography (6 ml gel bed of P-6). The plasma samples were eluted with phosphate buffer, pH 7.4, and aliquants of 0.5 ml were collected. 125 I-labelled relaxin eluted at fractions 5 and 6, and free iodide at fractions 12 and 13. Radioactivity from fragments of the polypeptide were recovered in fractions 8–11 (2 ml). Counts of these fractions were added and the ratios to fractions 5 and 6 were compared by a paired *t* test.

Tyrosine. L-Tyrosine was iodinated with chloramine-T as described above. Free iodide was separated from mono and di-iodinated derivatives by HPLC on a column (4.6 mm i.d., 250 mm length with guard column) packed with octadecyl-coated silica matrix (5 μ m o.d. Ultrasphere-IP, Altex, Palo-Alto, CA) and equilibrated with acetonitrile/0.05 M-phosphoric acid, pH 2.2 (10:90 v/v). Separation was achieved by elution with a linear gradient of ascending acetonitrile concentrations (0.5% CH_3CN /min). Only monoiodinated tyrosine was used for infusion experiments. Iodinated tyrosine was diluted 1:10 with the non-iodinated amino acid so that the final preparation had a specific activity of 0.14 μ Ci/pmol. The substance was neutralized with phosphate buffer and used within 3 days of preparation. Before infusions, organic solvent was completely evaporated with nitrogen and a filter sterilized (0.22 μ m Millipore filter) solution of 110 pg/ml in physiological saline (0.9%) was prepared.

Infusions. With one exception (in Sheep T3, Table 1), two infusion experiments were carried out in each sheep. The infusions extended over a period of 1 h and were separated by a rest period of 75 min. Substances were infused with a syringe pump at a rate of 100 μ l/min. To keep the physical

conditions for the two consecutive infusions similar, changes in the position of the sheep or in the arrangement of the infusion pump, syringe, tubings, and catheters were avoided. Relaxin was infused at a rate of 37 ng/min (5.8 pmol/min; 1.25 μ Ci/min) and tyrosine at a concentration of 1.1 ng/min (5.8 pmol/min; 0.83 μ Ci/min).

Peripheral blood was continuously collected through the jugular cannula by means of a fraction collector (1–1.5 ml in 3 min). Ovarian arterial blood was also continuously collected. For most of the samples, between 550 and 750 μ l were obtained within 3 min. Blood samples were centrifuged, prepared as aliquants (200 μ l) within 1 h of collection and counted for radioactivity within 5 h. For the relaxin experiments, remaining aliquants were stored at -20°C for chromatographic analysis of representative samples (highest concentration of ^{125}I) to compare breakdown products in peripheral and ovarian arterial plasma.

Radioactive substances were infused into an ovarian venous branch about 15–20 mm from the ovarian hilus (site 1, see Fig. 1). Blood was collected from an ovarian artery supplying the ovary (ovarian branch of the ovarian artery, OBOA, site 2 or 3), and/or a tubal branch of the ovarian

Fig. 1. Ovary with venous drainage and arterial supply. One artery is stippled in comparison to other arteries to demonstrate the different surface areas with the veins. OBOA: ovarian branch of ovarian artery. TBOA: tubal branch of ovarian artery. UBOA: uterine branch of ovarian artery. OBOV: ovarian branch of ovarian vein. TBOV: tubal branch of ovarian vein. UBOV: uterine branch of ovarian vein. (1) Infusion of radiolabelled substance into OBOV; (2) and (3) collection of arterial blood from OBOA; (4) collection of arterial blood from TBOA; (5) collection of arterial blood from UBOA.

artery (TBOA, site 4), and/or the uterine branch of the ovarian artery (UBOA, site 5). These arteries are in close apposition to veins originating from the ovary (ovarian branch of the ovarian vein, OBOV), the oviduct (tubal branch of the ovarian vein, TBOV), or the tip of the uterine horn (uterine branch of the ovarian vein, UBOV).

Results

Relaxin

The local exchange of relaxin was investigated in 10 infusion experiments two performed in each of 5 sheep. In addition to peripheral blood, samples were collected from 12 arterial branches, supplying the ovary (OBOA, $n = 5$), the oviduct (TBOA, $n = 3$), and the tip of the uterine horn (UBOA, $n = 4$).

Chromatographic analysis of peripheral plasma and plasma collected from branches of ovarian arteries did not reveal significantly different ratios (matched t test; $P = 0.08$) of ^{125}I -labelled relaxin and radiolabelled fragments. It therefore seems that any relaxin which passed vessels and adjacent tissue was transferred as intact molecules.

In all experiments, blood collected from all branches of ovarian arteries contained higher levels of relaxin compared to jugular venous blood (see Table 1; Fig. 2a). Analysis of variance of the average ratios of radiolabelled substance (ovarian artery/jugular vein, sampled at the same time) between the first and second infusion did not reveal significant differences ($P > 0.05$). Therefore, both infusions were treated as a single group and compared with ^{125}I ratios in different arteries (i.e. OBOA, TBOA and UBOA). Ratios of radioactivity (arterial:jugular) within the branches and amongst them were also not significantly different: the average ratio ($\pm$ s.d.) over a 1-h infusion period was $1.42 (\pm 0.35)$.

Occasionally, this ratio fluctuated during the infusion. It is not clear whether these fluctuations were caused by varying exchange rates (e.g. changing apposition of arteries and veins at the ovarian pedicle due to peristaltic movement), or variations in blood flow, or both. While elevations of relaxin over the total infusion period of 1 h were only minimal in some experiments (average ratio of 1.09 in Sheep R4, TBOA and UBOA, Fig. 2a), average radioactivity three times as high as in jugular blood could also be observed (Sheep R1, OBOA). The maximal ratios of radioactive counts between arterial and jugular plasma within a 3-min collecting period are shown in Table 1. Counts as much as 5 times higher in an ovarian arterial blood sample compared to the parallel peripheral sample were measured in some experiments (e.g. Sheep R1, Table 1).

Fig. 2. Ratios of radiolabelled (a) relaxin and (b) tyrosine between different branches of ovarian arteries (OBOA, TBOA, UBOA; see Fig. 1) and peripheral blood (jugular vein) as an average over the entire infusion period of 1 h. Each cluster of bars represents an experiment in one sheep (R1–R5; T1–T4). The ratios of two consecutive infusions for each artery are plotted. Ratios > 1 indicate a local transfer from the ovarian vein to adjacent arteries.

Table 1. Maximal local exchange of ^{125}I -labelled relaxin or ^{125}I -labelled tyrosine as a percentage of the totally infused amount of polypeptide in different branches of ovarian arteries (based on 4 ml/min blood flow through the ovary) and maximal ratio of radio-labelled relaxin between arteries and peripheral blood within a collection period of 3 min

Sheep*	Infusion	Artery sampled†	Max. % exchange	Max. arterial:jugular ratio	Sheep†	Infusion	Artery sampled‡	Max. % exchange	Max. arterial:jugular ratio
R1	1	OBOA	0.37	2.50	T1	1	OBOA	0.48	2.50
	2	OBOA	1.30	4.91		2	OBOA	0.48	1.30
	1	UBOA	0.12	1.88		1	TBOA	0.64	1.59
	2	UBOA	0.16	1.51		2	TBOA	1.81	1.81
R2	1	OBOA	0.23	2.60	T2	1	UBOA	0.53	2.36
	2	OBOA	0.40	1.50		2	UBOA	0.46	1.33
	1	TBOA	0.32	3.30		1	OBOA	2.13	4.32
	2	TBOA	1.20	2.20		2	OBOA	1.80	1.37
R3	1	UBOA	0.18	2.44	T3	1	UBOA	5.96	4.87
	2	UBOA	0.43	1.44		2	UBOA	9.69	2.54
	1	OBOA	0.47	1.94		1	OBOA	2.52	3.45
	2	OBOA	0.73	1.47		1	OBOA	1.58	2.96
R4	1	UBOA	0.65	2.07	T4	1	OBOA	1.42	3.70
	2	UBOA	0.95	1.65		2	OBOA	3.40	2.60
	1	OBOA	0.34	3.20		1	TBOA	0.31	1.68
	2	OBOA	0.16	1.40		2	TBOA	0.79	1.79
R5	1	TBOA	0.27	3.74		1	UBOA	0.74	4.71
	2	TBOA	0.10	1.24		2	UBOA	0.54	1.43
	1	UBOA	0.28	3.76					
	2	UBOA	0.10	1.16					
	1	OBOA	0.24	2.32					
	2	OBOA	0.71	1.67					
	1	TBOA	0.24	2.19					
	2	TBOA	0.20	1.23					

* Data are shown for five sheep each of which received two infusions of ^{125}I -labelled relaxin for 1 h which were separated by a rest period of 75 min.

† Data are shown for 4 sheep each of which received two infusions of ^{125}I -labelled tyrosine under the same conditions as mentioned above except Sheep T3 which received only one infusion.

‡ OBOA = ovarian branch of ovarian artery; UBOA = uterine branch of ovarian artery; TBOA = tubal branch of ovarian artery.

Fig. 3. Percentage of totally infused radiolabelled (a) relaxin and (b) tyrosine transferred directly from ovarian veins to adjacent arteries (see Fig. 1) as an average over the 1-h sampling period. The uterine branch of the ovarian artery (UB) in Sheep T2 was particularly convoluted and a high transfer of tyrosine was measured in this animal.

Fig. 4. Radioactivity measured in the jugular vein, ovarian branch of the ovarian artery (OBOA), and uterine branch of the ovarian artery (UBOA) during an infusion experiment with ^{125}I -labelled relaxin derivative (Sheep R3). The infusion periods are marked with horizontal bars. Blood samples were collected over periods of 3 min.

The percentage of ^{125}I -labelled relaxin exchanged to ovarian arteries calculated for an average blood flow of 4 ml/min (Janson *et al.*, 1983) varied from 0.1% up to 0.75% (Fig. 3; average for all arteries $0.22\% \pm 0.12\%$). These values were calculated as averages over the 1-h infusion period. At peak values measured for a 3 min collection period, up to 1.3% of the total amount infused into the ovarian vein could be recovered in an ovarian artery after subtracting the concentration from peripheral blood (Table 1).

Radioactive counts measured in the jugular vein, an ovarian branch of the ovarian artery (OBOA), and a uterine branch (UBOA) for the infusion experiment in Sheep R3 are shown in Fig. 4 as a representative example. Compared to peripheral plasma (jugular vein), radioactivity in the ovarian branch and the uterine branch of the ovarian artery was elevated during the infusion

period. The rise of ^{125}I -labelled relaxin derivative in the ovarian arteries occurred within 3–6 min after starting the infusion.

Tyrosine

Seven infusions of tyrosine were performed (2 each in 3 sheep and 1 infusion in 1 sheep; the latter animal developed breathing problems during the second infusion and the study was terminated). Blood was collected from 9 ovarian arteries (OBOA, $n=4$; TBOA, $n=2$; UBOA, $n=3$; Figs 2b & 3b). As in the previous experiment, ratios of radiolabelled tyrosine in arterial and jugular blood plasma calculated over the 1-h infusion period for the first and second infusion were statistically not different ($P > 0.05$). Likewise, ratios for the three branches of ovarian arteries (ovarian, tubal, and uterine branch) did not differ significantly. Although a remarkably high exchange to a uterine branch of the ovarian artery was observed (see Sheep No. T2, Fig. 2b), in two other sheep (Nos T1 & T4), a smaller amount of tyrosine was locally exchanged to an equivalent artery so that the variance within this group exceeded the variance between the groups. As an average ($\pm$ s.d.) for all infusion experiments, ^{125}I -labelled tyrosine was $1.69 (\pm 0.38)$ times higher in ovarian arteries compared to peripheral blood. This is similar to the relaxin transfer ($P = 0.07$).

The maximal exchange rate for a 3-min period (arterial:jugular peak, Table 1) ranged from 1.30 (Sheep T1, OBOA) to 4.87 (Sheep T2, UBOA) with an average of 2.57. The percentage of totally infused ^{125}I -labelled tyrosine exchanged to ovarian arteries over the 1-h infusion period (Fig. 3b; average for all arteries $1.2\% \pm 0.5\%$) was significantly higher than for relaxin ($P = 0.005$). The maximal amount locally exchanged to the organs within a 3 min period was on average 1.9% of the totally infused amount of tyrosine (Table 1) and was as high as 9.69% in one set of blood samples (Sheep T2, UBOA).

Although quantitatively difficult to measure, it seems that the common contact area between arteries and veins determines the exchange rate for a certain substance. This is expressed in the high variation of exchange in the same branches of different sheep (Figs 2 & 3) which reflects individual anatomical variations. In most of the experiments, elevated amounts of radiolabelled substance were detected within 3 min after onset of the infusion. After the infusions were terminated, in most cases a sharp decline in radioactivity followed (see Fig. 4). Only occasionally was a gradual decrease observed. The pattern of the exchange was similar for both substances.

Discussion

Two results of this study were unexpected. First, a substantial amount of relaxin is exchanged directly at the ovarian pedicle. Free & Jaffe (1975) could demonstrate local exchange for testosterone at the pampiniform plexus in the male rat which has a similar venous–arterial network but not for the polysaccharide methoxyinulin with a molecular weight comparable to relaxin. It therefore appears that molecular size alone does not determine whether a substance is transferred locally. This was supported by the second unexpected finding. The ratios of radiolabelled substance between ovarian arteries and peripheral blood for tyrosine (1.7) and relaxin (1.4) were not significantly different. Moreover, we have found an even higher exchange ratio for oxytocin (M_r 1007) in similar experiments (Schramm *et al.*, 1986).

The molar percentage of exchanged tyrosine was about 5 times higher than for relaxin. However, due to the difference in molecular weight for the two substances, relaxin was infused at a rate of 37 ng/min and tyrosine at a rate of 1.1 ng/min (5.8 pmol/min for each substance). For relaxin, the organs supplied by ovarian arteries are perfused with 81 pg/min above peripheral concentration while for tyrosine 13 pg/min are directly exchanged. In evaluating potential physiological effects of directly transferred substances, these considerations become important.

Besides molecular size and polarity of the substance, there are some indications that the efficiency of the transfer is also concentration dependent (Land, Baird & Scaramuzzi, 1976), i.e. the exchanged percentage is higher at low concentrations in ovarian venous blood. However, how general this phenomenon is still needs to be investigated since Einer-Jensen & Waites (1977) found the opposite tendency for counter-current transfer of testosterone in vessels of the spermatic cord in monkeys.

Whether local transfer of an amount $>1\%$ is of physiological significance needs to be established separately for each biologically active substance under consideration. For prostaglandin F-2 α which regulates luteal regression in sheep and other species (for review, see Horton & Poyser, 1976), it has been shown that an exchange of 0.1% to 0.6% is within the physiological range (Land *et al.*, 1976). Presumably, biologically highly active substances which are not stable in the peripheral circulation might have the most pronounced effect upon local transfer. In view of their instability, these are substances that are most difficult to detect in peripheral blood. These investigations indicate that polypeptides might be included into the list of potentially regulatory signals acting by local transfer.

In view of our results, the frequently mentioned suggestion that small molecular weight substances are more suitable for counter current transfer between veins and arteries seems unjustified. Rather, we suggest that passive exchange is governed by the partition coefficient of the exchanged substance between aqueous medium and tissues (solubility in blood, interstitial fluid, and in cell membranes; lipophilic/hydrophilic properties), and spatial size. This is only valid for substances that are not actively transferred, e.g. by receptor-mediated or specific channel mediated transport.

The amount of substances exchanged from veins to arteries is probably dependent on the common area between veins and arteries at the ovarian pedicle. This explains the high variation of radioactive counts in different animals. Thus, the individual anatomical structure at the ovarian pedicle in sheep or at the uterine adnexa in women (Bendz, 1977; Bendz, Einer-Jensen, Lundgren & Janson, 1979) might contribute to the physiological function of reproductive organs and this should be taken into consideration for diagnosis of some hormone related reproductive abnormalities.

The present and previous (Schramm *et al.*, 1986) studies have demonstrated that polypeptides and other molecules might be locally exchanged and can thus potentially act as regulators of endocrine function. Venous-arterial exchange of biologically active substances might also occur in similar structures of other species, e.g. the human uterine adnexa (Bendz, 1982) and the 'rete mirabilis' in the brain (see Baker, 1979).

We thank Tom On and Douglas D. Doop for preparing the animals and performing surgery; Mark D. Byrne for radiolabelling and purification of reagents. Relaxin was a gift from the National Pituitary Agency, NIH. The experiments have been supported by the Biomedical Research Council at The University of Michigan, and the Danish Research Council.

References

- Baker, M.A. (1979) A brain-cooling system in mammals. *Sci. Am.* **240**, 114–122.
- Bendz, A. (1977) The anatomical basis for a possible counter current exchange mechanism in the human adnex. *Prostaglandins* **13**, 335–362.
- Bendz, A. (1982) Countercurrent exchange in the human female reproductive tract. Ph.D.-Thesis. University of Goteborg, Sweden.
- Bendz, A., Einer-Jensen, N., Lundgren, O. & Janson, P.O. (1979) Exchange of krypton-85 between the blood vessels of the human uterine adnexa. *J. Reprod. Fert.* **57**, 137–142.
- Coudert, S.P., Phillips, G.D., Faiman, C., Chernecki, W. & Palmer, P. (1974) A study of the utero-ovarian circulation in sheep with reference to local transfer between venous and arterial blood. *J. Reprod. Fert.* **36**, 319–331.
- Einer-Jensen, N. & McCracken, J.A. (1981) The transfer of progesterone in the ovarian vascular pedicle of the sheep. *Endocrinology* **109**, 685–690.

- Einer-Jensen, N. & Waites, G.M.H. (1977) Testicular blood flow and a study of the testicular venous to arterial transfer of radioactive krypton and testosterone in the rhesus monkey. *J. Physiol., Lond.* **267**, 1–15.
- Free, M.J. & Jaffe, R.A. (1975) Dynamics of venous-arterial testosterone transfer in the pampiniform plexus of the rat. *Endocrinology* **97**, 169–177.
- Ginther, O.J. (1976) Comparative anatomy of utero-ovarian vasculature. *Vet. Scope* **20**, 1–17.
- Horton, E.W. & Poyser, N.L. (1976) Uterine luteolytic hormone: a physiological role for prostaglandin $F_{2\alpha}$. *Physiol. Rev.* **56**, 595–651.
- Hunter, W.M. & Greenwood, F.C. (1962) Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature, Lond.* **194**, 495–496.
- James, R., Niall, H.D., Kwok, S. & Bryant-Greenwood, G.D. (1977) Primary structure of porcine relaxin: homology with insulin and related growth factor. *Nature, Lond.* **267**, 544–546.
- Janson, P.O., Williams, D., Petrucco, O.M., Amato, F., Seamark, R.F. & Findlay, J.K. (1983) Blood flow in the ovary and adjacent structures of the non-pregnant sheep. *Acta endocr., Copenh.* **103**, 259–265.
- Land, R.B., Baird, D.T. & Scaramuzzi, R.J. (1976) Dynamic studies of prostaglandin $F_{2\alpha}$ in the utero-ovarian circulation of the sheep. *J. Reprod. Fert.* **47**, 209–214.
- McCracken, J.A., Schramm, W. & Einer-Jensen, N. (1984) The structure of steroids and their diffusion through blood vessel walls in a counter-current system. *Steroids* **43**, 293–303.
- Porter, D.G. (1979) Relaxin: old hormone, new prospect. In *Oxford Reviews of Reproductive Biology*, pp. 1–57. Ed. C. A. Finn. Clarendon Press, Oxford.
- Schramm, W., Einer-Jensen, N., Schramm, G. & McCracken, J.A. (1986) Local exchange of oxytocin from the ovarian vein to ovarian arteries in sheep. *Biol. Reprod.* in press.
- Schwabe, C., McDonald, J.K. & Steinetz, B.G. (1977) Primary structure of the B chain of porcine relaxin. *Biochem. Biophys. Res. Commun.* **75**, 503–510.
- Wathes, D.C. (1984) Review. Possible actions of gonadal oxytocin and vasopressin. *J. Reprod. Fert.* **71**, 315–345.
- Wood, F.T., Wu, M.M. & Gerhart, J.C. (1975) The radioactive labelling of proteins with an iodinated amidination reagent. *Analyt. Biochem.* **69**, 339–349.

Received 16 October 1985