Journal of Steroid Biochemistry, 1974, Vol. 5, pp. 969–975. Pergamon Press. Printed in Great Britain.

ESTROGEN RECEPTORS IN THE PANCREAS

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SUMMARY

Minced *rat* pancreas retained a large portion of estradiol (E_2) after incubation with either 5 nM or 1 μM concentrations of radioactive E_2. The mince was homogenized in low salt buffer and separated into nuclei, mitochondria, microsomes, and cytosol by centrifugations at 1000 *g*, 10,000 *g*, and 105,000 *g*, respectively. The three pellets were washed 3 times prior to extraction with 0·4 M KCl buffer.

The minced tissue took up about 77% of the labeled E_2 irrespective of the initial concentration of E_2 in the incubation medium. The largest amount of radioactivity was retained by the microsomes; and no more than half of this radioactivity could be extracted with 0·4 M KCl. The distribution among the fractions was the same for incubations in 5 nM and 1 μM E_2. The ratio of E_2 to protein in the cytosol, and in the nuclear, mitochondrial, and microsomal extracts was of the order of magnitude of pgE_2/mg protein for incubations in 5 nM E_2, and of the order of magnitude of ngE_2/mg protein for incubations in 1 μM E_2, with the highest ratio in the microsomal extract. Similarly, percentage binding is not reduced by incubations in a 200-fold higher concentration of E_2. Binding was significant in all three extracts or cytosol. The sucrose density gradient gave higher results for binding than the assay by a Sephadex G-25 column. Per cent bound was more than 50% in all fractions as measured by sucrose density gradient, with the cytosol showing 90% binding. The bound radioactivity in cytosol and in the three extracts sedimented in the 4*s* region. The experiments show that rat pancreatic tissue has a high capacity to bind estrogens.

Binding curves with E_2 or estriol (E_3) were obtained with either *human* or *dog* pancreatic cytosol by equilibrium dialysis or DCC. These curves showed that the cytosol has a low capacity with an apparent association constant of 10^7 to 10^9 M^{-1} for E_2 and E_3 and a high capacity of low association, especially for E_2. A protein concentration effect was noted for the binding of E_2 or E_3 by human cytosol. Whereas only one peak in the 4*s* region was detected by sucrose density gradient, 2 or 3 peaks were obtained by chromatography of human or dog cytosol on Sephadex G-100. These results point to the possible presence of more than one E_3 binding in the pancreatic cytosol.

INTRODUCTION

The presence of specific "receptor proteins" for reproductive steroid hormones in so-called "target organs" (uterus, prostate, breast, seminal vesicles) has been well established and extensively studied[1–5].* Generally, the effects of these steroid hormones in tissues containing the receptor proteins are induced by relatively small amounts of these steroids, which produce evident and rather dramatic metabolic and anatomic alterations in the target tissue in a short time period, without the steroids undergoing any apparent molecular changes during this process. The effects of these steroids are allegedly dependent on the presence of a receptor protein in the cytoplasm in the 4*s* range, which combines specifically with the steroid, e.g. E_2 in the uterus, DHT in the prostate, and undergoes an orientational change, followed by interaction with sites on or associated with the nucleus, which leads to a series of events mediated by messenger RNA originating from transcription of the nuclear DNA and other RNA's and followed by synthesis of appropriate proteins.

More recently, receptor proteins for reproductive steroid hormones with many and sometimes all of the characteristics of those present in reproductive tissues have been described in organs not considered to be reproductive in nature, e.g. E_2 and DHT in the kidney [6, 7] and E_2 and E_3 in the pancreas[8]. The latter was demonstrated in our laboratory in the pancreas of the dog, baboon and human.

Studies in our laboratory pointed to the possible presence of receptor proteins for estrogens when it was shown that following the administration of either labeled E_2 or E_3 relatively more of the radioactivity was taken up by the pancreas of male dogs, rats and baboons than by any other organ or tissue[9–12].

* It is beyond the scope of this paper to refer to the large number of excellent studies dealing with this field and the reader should consult the papers by many authors presented in the volumes given as references[1–3] and two recent comprehensive reviews[4, 5].

Further studies showed that the preponderant amount of radioactivity (more than 80%) in the pancreas was in the form of unchanged E_2 or E_3 and that no such localization of labeled testosterone, DHT, or cortisol occurred in the gland[13]. Subsequent *in vitro* studies showed that incubation of the pancreatic tissue of dog, baboon or human leads to the association of a cytoplasmic receptor protein in the pancreas of these species with either E_2 or E_3, without any evident metabolic changes or conjugation of the steroid[8]. The pancreatic receptor proteins in the dog appeared to have many of the characteristics of the receptor proteins for estrogens in the uterus and other estrogenic target organs. Some evidence had existed for many years for the localization of administered estrogens, shown by autoradiography, primarily in the acinar cells of the rodent pancreas[14]. In recent studies we have obtained similar results with the dog pancreas.

The present report deals with the binding characteristics of cellular proteins for E_2 and E_3 in the *rat* pancreas and some further studies on the binding of E_2 and E_3 by dog and human pancreatic cytosol proteins.

EXPERIMENTAL

Adult male Wistar rats were used for the studies. The animals were anesthetized with ether and killed by cervical dislocation. Estradiol-17β-2,4,6,7 (N)-^{3}H and estriol-2,4,6,7 (N)-^{3}H with specific activities of 105 and 109 μCi/nmole, respectively, were purchased from the New England Nuclear Corporation.

The following buffers were used in the studies: TK-8·2 (0·01 M Tric–HCl, 0·15 M KCl, 0·02% NaN_3; pH 8·2), TK (0·01 M Tris–HCl, 0·15 M KCl, 0·02% NaN_3; pH 7·4), TE (0·01 M Tris–HCl, 0·0015 M Na_2EDTA, 0·02% NaN_3; pH 7·4), TEK (0·01 M Tris–HCl, 0·0015 M Na_2EDTA, 0·4 M KCl, 0·02% NaN_3; pH 7·4).

The methods used in determining binding of steroids, the various calculations involved and the determinations of radioactivity have been described previously [14, 15].

Equilibrium dialysis was done in 8 mm flat width dialysis casing (Union Carbide Corp.) with a 1 ml sample inside the casing and 3 ml of TK-8·2 buffer outside for 40 h at 4°C on a rotating drum at 15 rev/min. Absorption with dextran-coated charcoal (DCC) was performed at 4°C. A suspension of 0·5% Norit A and 0·005% Dextran C (6–9 × 10^4 mol wt) in 0·15 M KCl buffer was mixed with an equal volume of the sample, shaken and allowed to stand for 10 min and then centrifuged at 1000 *g* for 10 min. Filtration on Diaflo PM 10 membranes was done at a pressure of 50 psi in a model 52 filtration chamber of Amicon Corp. Sucrose density gradient (SDG) was performed with a 5–18·5% gradient for 20 h in a Beckman Ultracentrifuge (model L2-65B) with a SW41 rotor at 41,000 rev./min. Binding on Sephadex G-25 was performed in a Pasteur pipette with a void volume of 1·4 ml at a flow rate of 0·25 ml/min. Fifty λ of the sample was mixed with 50 λ of 40% sucrose plus 0·3% Dextran Blue 2000 in appropriate buffer (TE or TEK) and then applied on top of the G-25 with a Hamilton syringe. Details of the chromatography on Sephadex G-100 are in the legends to the figures.

The surgically removed pancreases of rats were immediately rinsed with ice cold saline and the tissue minced at room temperature. The mince was incubated with intermittent swirling at 35°C for 20 min or at room temperature for 30 min in RPMI no. 1640 tissue culture medium (1:1 by volume) containing 5 nM or 1 μM of labeled estradiol-17β (E_2) or estriol (E_3). All procedures thereafter were carried out at 0–4°C. After incubation the mince was centrifuged at 600 *g* for 10 min and the pellet then washed successively twice with two volumes of TK buffer and once with TE buffer by resuspension and recentrifugation at 600 *g*. The pellet was then homogenized with an equal volume of TE buffer in an ice-bath in the cold-room with a Potter–Elvehjem homogenizer with a motor-driven pestle intermittently for 1 min at a time interrupted by 1 min cooling periods. The homogenate was then centrifuged at 1000 *g* for 10 min to bring down the nuclei and the pellet re-suspended 3 times in two volumes of TE buffer and once in two volumes of TEK buffer and recentrifuged at 1000 *g* for 10 min after each suspension. The supernates of the TE suspensions were considered the 'washes' and those from the TEK the 'extracts'. The supernatant from the original 1000 *g* centrifugation was centrifuged at 10,000 *g* for 20 min to sediment the mitochondria, which were washed and extracted in the same manner as the nuclei. The supernate of the 10,000 *g* centrifugation was then centrifuged at 105,000 *g* for 75 min to bring down the microsomes. The latter were washed and extracted with TE and TEK buffers as above. The supernate of the 105,000 *g* centrifugation, known as the cytosol fraction, and the various washes, extracts and remaining pellets of the tissue were examined for their radioactive content.

The cytosol fraction of human or dog pancreas was prepared by rinsing the tissue with cold saline (0–4°C) immediately after obtaining the organ, which was then minced in the cold-room and then homogenized as above in an equal volume of 0·15 M KCl buffer. The homogenate was centrifuged at 1000 *g* and then the supernate centrifuged at 105,000 *g*. The latter supernate was considered to be the cytosol fraction of the pancreas.

Table 1. Radioactivity of E_2 associated with various cellular fractions of rat pancreas (expressed as per cent of radioactivity in the homogenate)

Differential centrifugation	% Radioactivity* Supernate	Wash	Extract	Pellet	Recovery
1000 *g*	76·8	19·1	2·8	0·8	99·5
10,000 *g*	60·2	8·3	0·7	1·4	70·6
105,000 *g*	8·2	11·5	2·3,† 19·4‡	20·7	42·7,† 59·8‡

* Average of 8 or 10 determinations, i.e. 4 or 5 for each of the two concentrations (5 nM or 1 μM) of E_2 in the incubation medium.
† Incubation at room temperature, average of 4 determinations.
‡ Incubation at 35°C, average of 4 determinations.

RESULTS

Incubation of rat pancreas with E_2

About 77% of the labeled E_2 became associated with the minced pancreatic tissue irrespective of the concentration of E_2 in the incubation medium. The relative distribution of E_2 into the subcellular fractions was also found to be independent of the concentration of E_2 in the incubation medium. Table 1 gives the distribution of E_2 in the subcellular fractions. The temperature of incubation has marked effect only on the per cent E_2 extractable from the microsomal pellet. That this difference in the per cent extractable is not related to the recoveries, which are within the experimental errors, can be seen from the results given in Table 2. A marked difference in per cent bound was noted only for the microsomal extract as measured by chromatography on Sephadex G-25 and by SDG.

On the basis of the protein concentration (Lowry protein expressed as mg BSA) the various cellular fractions appeared to have the same capacity for binding E_2, except for the microsomes which bound an inordinately large percentage of E_2 after incubations of minced tissue with E_2 at 35°C (Table 2). These *in vitro* data with rat pancreas demonstrate the high capacity of this tissue for estrogens, with the largest amount of E_2 being found in the microsomal fraction.

Identical experiments are currently being carried out with E_3. Preliminary results show that the subcellular distribution of E_3 in rat pancreas is very similar to that of E_2. However, the per cent E_3 bound in the cytosol and microsomal extract is less than one half of the per cent E_2 bound as determined by Sephadex G-25. Only negligible binding was found in the nuclear and mitochondrial extracts.

Experiments with human and dog pancreatic cytosol

Binding curves were obtained by either equilibrium dialysis or DCC. They showed that the cytosol has a low capacity with a high apparent association constant (K_{ass}) for E_2 or E_3 and a high capacity with yet unknown, low association constants (non-specific binding). The K_{ass} were derived from the initial slopes of the b/u* as a function of $[b]$* for points of large b/u and low $[b]$. Some of the K_{ass} obtained are summarized in Table 3.

When fresh human cytosol was used, the apparent K_{ass} were slightly lower in this experiment than the association constants obtained on the same cytosol when stored in the cold-room for three weeks and diluted 1:20. This discrepancy may be the result of a concentration effect as shown in Table 5. In both cases, high apparent K_{ass} were obtained. The K_{ass} for E_3 were one order of magnitude higher than those for E_2.

The binding of fresh dog cytosol was determined by DCC and the results indicated a high K_{ass} for E_3, with a typical binding curve. A similar experiment with E_2 did not yield a satisfactory curve and the results are not shown in Table 3. K_{ass} was often difficult to determine, probably because of the large capacity for large

* $[b]$ = concentration of bound estrogen; b/u = ratio of bound to unbound estrogen.

Table 2. Uptake per Lowry protein and per cent binding of radiolabeled E_2 in rat pancreas*

Cellular fraction	ng E_2/mg protein Incubation with 5 nM E_2	Incubation with 1 μM E_2	%*b* G-25	SDG
Nuclear extract	$4·4 \times 10^{-3}$	1·1	14·3	59·3
Mitochondrial extract	$4·7 \times 10^{-3}$	2·0	8·4	75·3
Microsomal extract	$2·1 \times 10^{-3}$† $54·5 \times 10^{-3}$‡	1·6,† 9·5‡	0·1,† 60·1‡	25·1,† 95·9‡
Cytosol	$4·3 \times 10^{-3}$	2·1	57·3	90·5

*, †, ‡ see footnotes to Table 1.

Table 3. K_{ass} of E_2 and E_3 in cytosol and its Sephadex G-100 fraction

Material and conditions	K_{ass} in M^{-1} E_2	E_3
Human cytosol (2/23) equil. dial. same day	7×10^7	5×10^8
Human cytosol (2/23) equil. dial. 3 weeks in cold room 1:20 dilution	3×10^8	4×10^9
Dog cytosol (3/20) DCC same day		3×10^9
Dog cytosol (10/4) G-100, peak I equil. dial.		3×10^8
Dog cytosol (5/1) G-100, peak I/II lyophilized equil. dial.		2×10^7
As above DCC		2×10^7

'nonspecific' binding especially of E_2, and a protein concentration effect (Table 5).

In another experiment, the binding data obtained for E_3 from the cytosol of another dog could not be evaluated for K_{ass}; the slope for $[b]$ as a function of (b/u) was positive, i.e. K_{ass} was negative. However, after chromatography of the same cytosol on Sephadex G-100 (Fig. 1), a well-defined binding curve was obtained with a high K_{ass} as shown in Table 4.

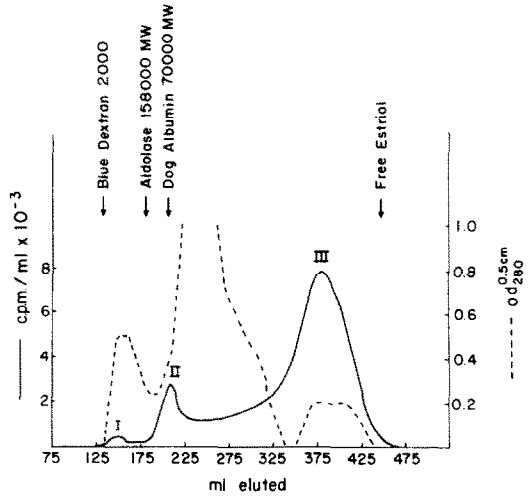

Fig. 1. Fractionation of cytosol of dog (10/4) pancreas after incubation for 20 h at 4°C with tritiated estriol (E_3) on Sephadex G-100: column height 77 cm, diameter 2·5 cm, flow rate 0·167 ml/min. Eluting buffer—same as homogenizing buffer—0·01 M Tris–HCl, 0·15 M KCl, 0·02% NaN_3; pH 8·2.

Table 4. Equilibrium dialysis of E_3 with dog (10/4) cytosol and peak I following Sephadex G-100 chromatography

Material used	$[b]$ (pM)	$\frac{b}{u}$	Apparent K_{ass} in M^{-1}
Cytosol	0·37	7·8	Negative
	11·9	8·7	
	129	11·6	
	1230	11·6	
	12,300	11·8	
Peak I	5·6	15	$\sim 3 \times 10^8$
	2800	17	
	24,300	9·4	
	215,000	6·5	
	1,770,000	4·2	

The cytosol of the pancreas of another dog was chromatographed on a G-100 column and the peaks containing the radioactivity were lyophilized. The latter did not destroy the binding ability. The binding was higher with equilibrium dialysis than with DCC, since equilibrium dialysis measures *all* binding parameters, whereas DCC measures binding with a high K_{ass}. As expected, the K_{ass} value was the same with both techniques.

The concentration effect of the binding of E_2 or E_3 in the cytosol of human pancreas was determined either by multiple equilibrium dialysis, in which bags with varying concentrations of cytosol were dialyzed in the same flask, or by individual dialysis. The results

Table 5. Binding of labeled E_2 or E_3 by human pancreatic cytosol of varying dilutions measured by multiple or separate equilibrium dialysis

Material used	Cytosol dilution	d.p.m. bound/ml × dil. of cytosol	b/u × dil. of cytosol
E_2 Multiple dialysis	1:0	23,700	1·8
	1:1	30,000	2·3
	1:9	30,900	2·4
	1:25	34,900	2·7
	1:100	37,400	2·8
E_3 Multiple dialysis	1:0	10,100	7·9
	1:1	14,000	10·9
	1:9	28,700	22·4
	1:25	24,600	19·2
	1:50	29,200	22·3
E_2 Separate dialysis	1:0	88,000	5·6
	1:1	171,000	10·1
	1:9	402,000	14·3
	1:49	397,000	11·5
E_3 Separate dialysis	1:0	6900	2·3
	1:1	8900	2·3
	1:9	19,400	4·2
	1:49	32,600	6·5
	1:99	55,900	11·0

are summarized in Table 5. They show a concentration effect of the binding protein to a varying degree.

In the multiple dialysis experiment, constant values in the second and third columns in Table 5 would have indicated absence of a protein concentration effect. An analysis of the data from the separate equilibrium dialysis show a positive slope of b/u vs d.p.m. bound, which is indicative of a protein concentration effect.

Fractionation of human or dog cytosol on Sephadex G-100 resulted in 2 or 3 radioactive peaks (Figs. 1 and 2), whereas only one peak in the 4s region was noted on sucrose density gradient. The first and smallest radioactive peak (peak I) is eluted close behind the void volume. A second peak (peak II) elutes close to dog serum albumin. Where 2 peaks were obtained, the first peak (peak I/II) was located in front of the dog albumin peak. The third peak (peak III) is eluted in front and together with free E_3. It is not unbound E_3 exclusively, as can be demonstrated by binding measured by equilibrium dialysis or DCC. The per cent of E_3 bound in Peak I of the chromatogram shown in Fig. 1 was about 10 × the per cent bound in peaks II and III, when measured by DCC (35·3, 3·6, and 4·6, respectively). The E_3 binder(s) of the radioactive peaks after chromatography on Sephadex G-100 can be concentrated on a Diaflo membrane PM10 with a nominal cutoff of 10,000 mol. wt. However, the losses of an E_3 binder into the filtrate are considerable. The cytosol of the chromatogram of Fig. 2 was treated on a Diaflo PM 10 filter prior to the application to the Sephadex G-100 column. This treatment reduced the concentration of low molecular weight species as reflected in the small area of peak III.

Further work on the fractionation of the E_3 binders and their characterization is in progress.

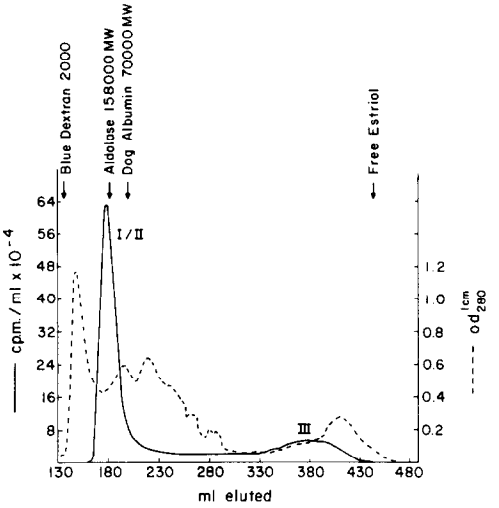

Fig. 2. Fractionation of cytosol of dog (8/9) pancreas on Sephadex G-100. The cytosol was diluted to 6 × its volume with buffer and concentrated to its original volume under pressure over a Diaflo PM10 filter. The treated cytosol was incubated with tritiated E_3 overnight at 4°C prior to fractionation on a Sephadex G-100 column with a height of 77 cm, diameter 2·5 cm, and flow rate of 0·163 ml/min. Eluting buffer—same as homogenizing buffer—0·01 M Tris–HCl, 0·15 M KCl, 0·02% NaN_3; pH 8·2.

DISCUSSION

The studies with rat pancreas demonstrated a high capacity of this tissue for estrogens, with the largest amount of E_2 and E_3 being found in the microsomal fraction. It was shown that all subcellular fractions of the rat pancreas, i.e. nuclei, mitochondria, microsomes, and cytosol bound considerable amounts of E_2, with more than 50% being bound by each fraction as measured by sucrose density gradient. Cytosol and microsomal extract actually showed 90% and 96% binding, respectively. The binding radioactivity sedimented in the 4s region in all extracts and cytosol. Interestingly, the per cent of labeled E_2 bound was not influenced by a 200-fold increase in the concentration of E_2 in the incubations and indicates a very high capacity by the pancreas of the rat to bind estrogens.

We also undertook an evaluation of receptor proteins for estrogens in the pancreas of the dog and human. Thus, it was shown that the pancreatic cytosol of the dog and human contained a binder with a limited capacity for the binding of E_2 but with a high K_{ass} of 10^7–10^9 M^{-1}, comparable to the constants of other estrogenic receptors[1–5]. In addition, another binder of high capacity for E_2 and E_3, but with as yet an unknown but probably low K_{ass} value was also present in the pancreas of the dog and human. The K_{ass} for the binding with the limited capacity has been difficult to establish probably due to (1) the large capacity of the less strongly binding sites and (2) a protein concentration effect. Several different approaches in our experiments indicated a definite protein concentration effect, accounting for some of the unusual results obtained. A protein–protein interaction has been described for receptors of calf thymus for cortisol[16], similar to our findings given in Table 5. It is interesting to note that whereas sucrose density gradient gave radioactive peaks in the 4s region with the cytosol and in the extracts of mitochondria, microsomes and nuclei, 2–3 peaks were obtained with dog or human cytosol and E_3 on Sephadex G-100. Thus, the results point to the possible presence of more than one type of binding protein in the pancreatic cells of the dog or human or the possible alteration of a binding protein due to a concentration effect.

The possible role estrogens play in the physiology of the pancreas is unknown. The demonstration of

receptor proteins for estrogens in the kidney and their physiologic role has been explained on the basis of the effect of these steroids on sodium and other electrolyte movements within the kidney[6]. Since the pancreas is also involved in shifts of electrolytes between it and its secretion into the gastrointestinal tract, it is possible that the estrogens play a role in that area also. However, it cannot be ruled out that estrogens may have a profound effect on other functions of the pancreas, particularly those related to the synthesis of proteins including various gastrointestinal enzymes. Studies in our laboratory are addressing themselves to solving some of the problems just enumerated.

Acknowledgements—This study has been supported in part by a grant (AM-01240) from the National Institutes of Health.

We wish to thank Mrs. Barbara Boehm and Mr. Michael Neylan for technical help and Miss Christine Hanson for clerical assistance.

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DISCUSSION

Korenman:

I was just wondering about proteolytic enzymes in the preparation. How do you work with them?

Sandberg:

The question as to the effects of the various proteolytic and other enzymes in the pancreas on the receptor and other binding proteins is not easily answered. However, we have found that the binding proteins are stable in the cold (5°C) for about 2 weeks; however, the so-called specific binder appears to be rather labile, which may be due to the effects of the proteolytic enzymes present in the pancreas. This lability is inconsistent, though, even when inhibitors of enzymes (e.g., iodoacetate) were added. Freezing and thawing led to considerable destruction of all binding systems.

Schrader:

I'm curious whether or not by any chance you've ever looked to see what cells might be involved. For example, do alloxan-treated rats show the same thing?

Sandberg:

We have not studied alloxan-treated rats. Since the pancreas consists of a number of different cells types, it would be very important to know in which cells these binding proteins are present. We have approached this by either exposing *in vitro* the pancreatic tissue of the dog or rat to tritiated estradiol-17β or injecting *in vivo* this steroid into the artery leading to the pancreas in the rat. The tissues are then processed for autoradiography and the location of the grains determined. In each case, most of the radioactivity was found to be in the acinar tissue. A search of the literature revealed that in 1963, Ullberg and Bengtsson (*Acta endocr.* **43**, (1963) 75), while studying the localization of radioactive estrone and estradiol in rodents found, to their surprise, high amounts of radioactivity localized in the pancreas, particularly in the acinar cells.

Schrader:

I have a comment which might be of interest as far as your having problems with recovery after the ultrafiltration. Amicon PM and UM series of ultra-filters binds steroids and so do the Centri-flo membranes. The only Amicon filters that are good to use with steroids are the XM series which don't bind. When you use the PM-10 it may just be that the only reason you saw bad recovery was because the steroid was sticking to it.

Martini:

I wonder whether you have studied the submaxillary gland of the rat as well. This because there are certain similarities, anatomically speaking, between the pancreas and the submaxillary gland. This is a gland which is certainly sexually di-morphic in rodents and the data I presented at this meeting indicate that this structure contains the 5α-reductase. We used the pancreas as control tissue for the submaxillary gland and, much to our surprise, we found that the pancreas

has a 5α-reductase activity. This means that androgens may also be influential in the pancreas. I wonder whether you have made the reverse experiment and studied the submaxillary gland.

Sandberg:

The submaxillary gland is definitely related embryologically to the pancreas. Hence, we did examine the submaxillary gland in the dog and found that there was no localization of estrogens in this particular tissue.

Kuss:

Inhibitors of proteases cannot be released from the enzyme like the substrates if an essential chemical step doesn't occur. Can it be possible that at least some of the so-called receptors are also some kind of wrong enzymes which do not release substrate and have nothing to do with the mechanism of hormone action?

Sandberg:

You're saying that the estrogen combines with an enzyme of some sort and that enzyme fails to release the estrogen and we're really measuring enzymes for which estrogens are substrates. Is that correct?

Kuss:

Estrogens were proposed to be not the correct substrates but substrates which cannot be released from the enzyme.

Sandberg:

It is possible that some of the binding could be due to such an interaction with enzymes, though it would be difficult to establish this particular facet of the interaction between estrogens and the proteins in the pancreas.

Rao:

Dr. Sandberg, I was wondering whether you have looked into any of the physical properties of the estrogen receptor protein that you eluted from the column and lyophilized. I was wondering if it could have aggregated or the molecule had changed during the isolation procedure.

Sandberg:

In general, some of the columns did give us difficulty in that possible protein–protein association leading to aggregates may have occurred. The G-100 column usually did not lead to such aggregation and, hence, we relied on that particular approach more often than others. However, we have not studied in any great detail the physical properties of the estrogen receptors following their elution from the columns, since we have found that the various binding parameters had not changed significantly from those obtained prior to the application of the materials to the columns.