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Composition of the inner mitochondrial membrane of porcine corpus luteum

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An inner mitochondrial membrane fraction was prepared from porcine corpus luteum. The concentrations of the respiratory cytochromes, cytochrome *P*-450$_{scc}$, cholesterol, ubiquinone, cardiolipin and the total phospholipids were measured. The fatty acid compositions of cardiolipin and the total phospholipid fraction were determined. Comparative data from porcine heart and liver were obtained using the same methods. Differences in both the concentration and the fatty acid composition of the phospholipids were observed between the tissues. It appeared that the phospholipid bilayer was expanded relative to haem *a* in luteal mitochondria. It is proposed that in the ovary this expansion may be necessary to accommodate cytochrome *P*-450$_{scc}$ and its substrate, cholesterol.

Introduction

The function of the inner mitochondrial membrane as an osmotic barrier and the activities of the membrane associated proteins are highly dependent on specific lipid–lipid and lipid–protein interactions within the membrane [1]. The inner membrane of most tissues is composed of approx. 75% protein and 25% lipid. Much of the protein is located external to the lipid bilayer so rather than being crowded, membrane proteins may be free to diffuse laterally in the plane of the membrane [2]. Considerable interest has been shown in the role of diffusion in mitochondrial electron transport [3–6].

The inner membrane of corpus luteum is of particular interest since it not only contains the ubiquitous respiratory electron transport chain, but also the cholesterol side chain cleavage enzyme, cytochrome *P*-450$_{scc}$, a second terminal oxidase which accepts electrons from a short soluble electron transport chain. These two electron transport chains probably draw electrons from the same source. Indeed evidence from several laboratories has shown that side chain cleavage activity can be regulated by altering the flux of electrons through the respiratory electron transport chain [7,8]. Conversely, ATP synthesis can be stimulated by inhibiting cytochrome *P*-450$_{scc}$ [9].

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In ovary the in vivo electron donors for both ATP synthesis and cholesterol side chain cleavage are probably fatty acids stored as cholesterol esters and triacylglycerols in cytoplasmic lipid droplets [10,11]. Activated fatty acids donate electrons to the respiratory electron transport chain via FAD-linked dehydrogenases. Robinson and Stevenson showed that both fatty acids [11] and succinate [12] can support side-chain cleavage in an energy dependent manner which does not involve reversed electron flow through respiratory complex I, but rather involves a direct energy-dependent link between acyl-CoA or succinate dehydrogenase and $NADP^+$. The nature of this link has yet to be established.

Although the composition of inner mitochondrial membranes of tissues such as rat liver and beef heart has been studied widely, there is much less information available for steroidogenic tissues such as the corpus luteum. As part of an investigation into the link between the respiratory and steroidogenic electron transport chains, we have examined the composition of the inner mitochondrial membrane of porcine corpus luteum. We were interested in determining any differences from non-steroidogenic tissues which could be involved in accommodating the steroidogenic machinery of the corpus luteum. In particular we have examined the respiratory cytochromes; ubiquinone which, as an electron acceptor for a number of dehydrogenases, could be involved in linking the electron transport chains; cardiolipin, the phospholipid which

activates cytochrome $P\text{-}450_{scc}$; the cholesterol content and the phospholipid to protein ratio. This paper is a report of our findings.

Materials

Cytochrome *c* (horse heart type III), sodium AMP, dipotassium ATP, disodium *p*-nitrophenyl phosphate and the sodium salt of cardiolipin were obtained from Sigma, St Louis, MO, U.S.A. Fatty acid methyl esters were obtained from Nu Check Prep, Elysean, MN, U.S.A. and Supelco, Bellefonte, PA, U.S.A. 1-Palmitoyl-2-[1-^{14}C]palmitoylglycerophosphocholine and [4-^{14}C]cholesterol came from Amersham, Bucks, U.K. *N,O*-bis(trimethylsilyl)acetamide was purchased from Pierce, Rockford, IL, U.S.A. All solvents used were of spectral grade or were redistilled before use. Other reagents were of the highest analytical grade available.

Methods

Isolation of mitochondria

Porcine ovaries were collected from the abattoir and transported to the laboratory on ice. Corpora lutea were dissected out and washed with ice-cold buffer A consisting of 0.25 M sucrose, 10 mM Tris phosphate (pH 7.4). All subsequent steps were performed at 4°C. Washed corpora lutea were chopped fine and homogenised in 4 vols. of buffer A using five strokes of a motor driven teflon pestle. The homogenate was centrifuged at $750 \times g_{max}$ for 10 min. The supernatant was carefully removed and centrifuged at $9800 \times g_{max}$ for 20 min to pellet mitochondria. The crude mitochondrial pellet was resuspended in 1/4 of the original volume and centrifuged at $750 \times g_{max}$ for 10 min. The supernatant was removed carefully and centrifuged at $9800 \times g_{max}$ for 20 min. The resultant mitochondrial pellet was freed of any haemoglobin contamination by resuspending in 0.154 M KCl, 10 mM Tris phosphate (pH 7.4) and centrifuging at $9800 \times g_{max}$ for 20 min.

Preparation of the inner membrane fraction

Inner mitochondrial membranes were prepared by a modification of the method of Sottocasa et al. [13]. Mitochondrial pellets were resuspended in 10 mM Tris phosphate (pH 7.4) to a concentration of 7–10 mg protein per ml. The suspensions were incubated on ice for 20 min before 1/3 volume of 1.8 M sucrose containing 2 mM ATP and 2 mM $MgSO_4$ was added. After a further 20 min incubation the suspension was sonicated in 5-ml aliquots using 3×5 s bursts of a Branson Sonifier set on 3. Samples were kept on ice during sonication and allowed to cool for 15 s between each burst.

Each aliquot of sonicated mitochondria was layered onto 25 ml of 1.18 M sucrose and centrifuged at $30\,000 \times g_{av}$ for 3 h in a swinging bucket rotor. The upper yellow layer and the interface material were removed and the sucrose layer, which separated them from the pellet, was discarded. The pellet consisting of inner mitochondrial membrane vesicles was rinsed twice with buffer A and resuspended in the same buffer. The upper yellow layer and interface material were centrifuged at $108\,000 \times g_{max}$ for 1 h. The supernatant contained soluble mitochondrial components (mitosol) while the pellet comprised the outer mitochondrial membrane fraction.

Marker enzyme assays

Cytochrome *c* oxidase activity, a marker of the inner mitochondrial membrane, was assayed as previously described [14] except that the buffer used was 100 mM Hepes-KOH (pH 7.2), 200 mM KCl and 10 mM $MgCl_2$.

Alkaline phosphatase was used as a marker for the outer mitochondrial membrane [15]. As observed previously for the bovine corpus luteum, monoamine oxidase was not detected in this fraction. Rotenone-insensitive NAD(P)H–cytochrome-*c* reductase is an unsuitable marker for the corpus luteum since the ferredoxin reductase-ferredoxin (adrenodoxin reductase-adrenodoxin) couple can act as a rotenone-insensitive cytochrome-*c* reductase.

5′-Nucleotidase activity, using AMP as substrate, was used as a plasma membrane marker [16,17].

Measurement of cytochrome concentrations

The concentrations of respiratory cytochromes were determined from their reduced minus oxidised difference spectra as before [14]. Cytochrome $P\text{-}450_{scc}$ concentrations were determined from the reduced-CO minus reduced difference spectra using $\epsilon_{450\text{-}490} = 91\,000\ M^{-1} \cdot cm^{-1}$ [18].

Measurement of ubiquinone concentration

Aliquots of 0.5 ml of inner mitochondrial membrane suspensions were denatured with 2 ml of methanol and extracted with 4×2 ml of petroleum ether. The combined extracts were washed with 2 ml of 95% methanol and dried under N_2. The residues were redissolved in 3 ml of ethanol and the oxidised minus borohydride-reduced difference spectra recorded between 200 and 350 nm. Concentrations were determined using $\epsilon_{275\text{-}290} = 12\,200\ M^{-1} \cdot cm^{-1}$ [19].

Extraction and purification of cholesterol and phospholipids

Cholesterol was extracted, separated from other lipids by thin-layer chromatography and quantitated by GLC as described previously [20].

Phospholipids were extracted from suspensions of inner membranes as before [15]. The extracts were applied as bands to plates coated with a 0.5 mm thickness of Silica gel H. For purification of total phospholipids,

plates were developed with hexane/diethyl ether/acetic acid (80 : 20 : 1, v/v). Bands were visualised with iodine vapour and identified by comparison with standard lipids run on the same plate. The phospholipid band, which remained at the origin, was scraped from the plate and the phospholipids were extracted as described previously [15].

For purification of cardiolipin the plates were developed with petroleum ether/acetone (3 : 1, v/v) followed by chloroform/methanol/water (65 : 25 : 4, v/v). Bands corresponding to authentic beef heart cardiolipin were scraped and extracted as were total phospholipids. The extracts were applied to a second Silica gel H plate and developed with petroleum ether/acetone (3 : 1, v/v) followed by chloroform/methanol/acetic acid/water (80 : 13 : 8 : 0.4, v/v). A single band resulted which corresponded to authentic beef heart cardiolipin and this was extracted as above.

Methanolysis of the phospholipids was performed by the method of Tuckey and Stevenson [20]. The methyl esters were analysed using a Shimadzu GC-8A gas chromatograph fitted with an SGE unijector and a BP20 bonded phase, vitreous silica column (0.22 mm i.d. × 25 m). The carrier gas was helium with a flow rate of 0.5 ml/min and the column temperature was 180 °C. The methyl esters were identified by comparison of their retention times with those of standards and quantitated by comparison of the area under the peak with that of an internal standard, methyl heptadecanoate. Molar concentrations were calculated using the molecular weight of methyl octadecenoate and assuming four fatty acids per cardiolipin and two for the other phospholipids.

Protein concentration

Protein concentration was determined by the method of Pesce and Strand [21] using bovine serum albumin as standard.

Results

Marker enzymes

The recovery of marker enzyme activities is summarised in Table I. Recovery of cytochrome-*c* oxidase activity in the mitochondrial fraction of corpus luteum averaged 69% (range 58–75%). On fractionation of the mitochondrial membranes 63% (range 44–80%) of the mitochondrial activity was in the inner membrane giving an overall recovery of 43% with respect to the whole homogenate.

Recovery of alkaline phosphatase activity in the luteal inner membrane fraction averaged 1.0% (0.8–1.1%). Although alkaline phosphatase has been used previously as an outer mitochondrial membrane marker [15], it is also present in the endoplasmic reticulum. Its use in determining purity of the inner mitochondrial membrane is not impaired by its dual location. Recovery of mitochondrial alkaline phosphatase activity in the inner membrane fraction was 7.7% and this would represent a maximum value for contamination by outer membranes.

Recovery of 5′-nucleotidase activity in the inner membrane fraction averaged 2.9% (2.3–3.8%) indicating a low level of plasma membrane contamination. Similar results were obtained for these markers in porcine heart and liver preparations.

TABLE I

Recovery of marker enzyme activities

Porcine corpora lutea were subfractionated and enzyme activities determined as described under Methods. Values are the means of duplicate determinations from a single experiment in which the range was less than 5% of the mean. Similar results were obtained with three other preparations.

Enzyme fraction	Specific activity (nmol/min per mg protein)	Total activity (nmol/min per fraction)	Recovery (%)
1. Cytochrome-*c* oxidase			
Homogenate	97.8	88000	100
Nuclear pellet	70.7	12400	14.1
9800 × *g* supernatant	7.9	4400	5.0
Mitochondria	275	51300	58.3
Inner membrane	852	38400	43.6
Outer membrane	357	4500	5.1
Mitosol	42.2	700	0.8
2. Alkaline phosphatase			
Homogenate	17.0	15700	100
Nuclear pellet	25.7	4500	28.7
9800 × *g* supernatant	22.1	12400	79.0
Mitochondria	11.5	2200	14.0
Inner membrane	7.5	170	1.1
Outer membrane	53.7	1100	7.0
Mitosol	24.2	390	2.9
3. 5′-Nucleotidase			
Homogenate	6.4	5800	100
Nuclear pellet	9.0	1590	27.4
9800 × *g* supernatant	5.1	2870	49.5
Mitochondria	8.4	1570	27.1
Inner membrane	6.7	150	2.6
Outer membrane	38.2	570	9.8
Mitosol	37.4	600	10.3

Concentrations of cytochromes and lipids

The concentrations of the inner mitochondrial membrane components measured are shown in Table II. Results from a single experiment using pig heart inner membranes prepared in the same way are also included.

The concentrations of the respiratory cytochromes and ubiquinone were lower in the corpus luteum than in the heart which is a reflection of the high oxidative capacity of heart tissue. When the results were expressed relative to haem *a* content, the level of ubiquinone was similar in both tissues. The high relative

TABLE II

Concentrations of cytochromes and lipids of the inner mitochondrial membrane

The inner mitochondrial membrane fraction was prepared and the cytochrome and lipid concentrations were determined as described under Methods. Values for corpus luteum are means ± S.D. of duplicate determinations from three experiments. Values for heart are means of duplicate determinations from one experiment in which the range was less than 5% of the mean.

Inner membrane component	Concentration			
	corpus luteum		heart	
	nmol/mg protein	relative to haem *a*	nmol/mg protein	relative to haem *a*
Cytochrome				
$a + a_3$	0.345 ± 0.029	1.0	0.987	1.0
b	0.633 ± 0.097	1.8	0.919	0.9
$c + c_1$	0.481 ± 0.039	1.4	0.783	0.8
P-450$_{scc}$	0.722 ± 0.077	2.1	–	–
Ubiquinone	3.04 ± 0.24	8.8	7.97	8.1
Cardiolipin	31.5 ± 4.6	91.3	58.4	59.2
Total phospholipids	386 ±35	1120	545	552

concentration of cytochrome *b* in the luteal inner membranes is due to the presence of cytochrome P-450$_{scc}$, a *b*-type cytochrome which was not detected in heart. The relative concentrations of cytochromes $c + c_1$ were also higher in luteal inner membranes. This also could be due to the presence of cytochrome P-450$_{scc}$ since there is considerable overlap of the α peaks of the *b* and *c* cytochromes [22]. Alternatively it may reflect differential losses of the soluble cytochrome *c* in the sub-fractionation procedure or differences in the stoichiometry of the respiratory complexes between the two tissues.

To determine if the sub-fractionation procedure caused any differential losses of inner membrane components, cytochrome concentrations were determined on a batch of luteal mitochondria before and after removal of the outer mitochondrial membrane (Table III). The relative increase in concentration of cytochromes $a + a_3$, *b* and P-450$_{scc}$ were similar indicating no selective removal of these components. The relative increase in cytochromes $c + c_1$ was lower, however, indicating that the loss of soluble cytochrome *c* on sub-fractionation was approx. 20%.

The mean cholesterol content of the inner membrane of corpus luteum was 28.4 nmol/mg protein or 0.074 mol/mol phospholipid. There was a large variation in cholesterol content between individual experiments, however. Values of 47.5, 56.2, 17.6, 23.8, 11.6, 13.9 and 28.5 nmol/mg protein were obtained in separate experiments. No corresponding differences were observed in marker enzyme assays, so it is unlikely that this variation was due to different degrees of contamination by other membranes. More likely it is a reflection of the hormonal status of the animals. Similar variations in the cholesterol content of steroidogenic mitochondria have been noted previously in rat adrenal cortex [23].

The concentration of cardiolipin per mg of protein in the inner membrane of porcine luteal mitochondria was lower than that of porcine heart (Table II) as was the phospholipid concentration. However, when expressed relative to haem *a*, the cardiolipin concentration was 50% higher and the phospholipids double the concentration in heart. Cardiolipin represented 8.2 mol% of the luteal inner membrane phospholipids or 15.1% of the phospholipid phosphorus compared to values of 10.7 mol% and 19.3% of phospholipid phosphorus for heart. The heart value was slightly lower than the 25.4% of phospholipid phosphorus reported by Comte et al. [24] for porcine heart inner membranes, although their value included phosphatidic acid which was not detected in our system. The cardiolipin and phospholipid concentration and the percentage of phospholipid represented by cardiolipin in the porcine luteal inner membranes were similar to the values of 27 nmol/mg, 310 nmol/mg and 8.7 mol% reported by Tuckey and Stevenson [15] for the bovine luteal inner membrane.

Fatty acid composition of phospholipids

The fatty acid composition of the phospholipids of the inner mitochondrial membrane from porcine corpus luteum is listed in Table IV. Values for porcine heart and liver were determined using the same methods.

The major fatty acids of the inner membrane phospholipids were 16 : 0, 18 : 0, 18 : 1, 18 : 2 and 20 : 4. The fatty acid profiles were similar in the three tissues. The main exceptions were 16 : 0 which was higher in corpus luteum than in heart or liver; 18 : 2 which was higher in heart than in corpus luteum or liver and 18 : 0 which

TABLE III

Cytochrome concentrations in unfractionated mitochondria and inner mitochondrial membranes of porcine corpus luteum

Mitochondria and inner mitochondrial membranes were prepared and cytochrome concentrations determined on a single batch of tissue as described under Methods. Values represent the means of duplicate determinations: the ranges were all less than 5% of the means.

Cytochrome	Mitochondrial concentration (nmol/mg protein)	Inner membrane concentration (nmol/mg protein)	Relative increase in concentration on fractionation
$a + a_3$	0.231	0.312	1.35
b	0.566	0.739	1.31
$c + c_1$	0.487	0.525	1.08
P-450$_{scc}$	0.508	0.664	1.31

TABLE IV

Fatty acid composition of the phospholipids of the inner mitochondrial membrane

Phospholipids were extracted and purified and the fatty acid composition determined as described under Methods. Values for corpus luteum represent the mean of duplicate determinations from three experiments. Values for heart and liver represent the mean of duplicate determinations from a single experiment. In all cases the range was less than 5% of the mean.

Fatty acid	Composition, percent by weight		
	corpus luteum	heart	liver
14:0	0.07	0.57	0.00
15:0	0.00	5.75	0.00
16:0	17.15	11.19	10.39
16:1(9)	0.21	1.24	0.94
18:0	17.91	10.40	30.59
18:1(9)	12.70	15.54	10.11
18:1(11)	1.05	0.00	1.79
18:2(9,12)	19.49	27.38	18.45
18:3(6,9,12)	0.00	0.00	0.25
18:3(9,12,15)	0.13	0.21	0.43
20:2(11,14)	2.62	0.36	0.87
20:3(8,11,14)	0.65	0.00	1.26
20:4(5,8,11,14)	23.53	21.26	17.18
20:5(5,8,11,14,17)	0.32	0.00	1.31
22:3(13,16,19)	0.23	0.00	0.00
22:4(7,10,13,16)	2.37	0.00	0.57
22:5(7,10,13,16,19)	0.72	1.29	2.27

varied the most, rising from 10.4% in heart to 17.4% in corpus luteum and 30.6% in liver.

Acids 20:2 and 22:4 were significant minor components of the corpus luteum but were absent, or present at very low levels in heart and liver, while 22:6 was present in liver at higher levels than corpus luteum or heart.

The fatty acid composition of porcine luteal inner membrane phospholipids was very similar to that of bovine corpus luteum calculated from the data of Tuckey and Stevenson [15]. The one exception was 20:4 which comprised 11.4% in bovine tissue as opposed to 23.5% in this study. We also found a higher level of 20:4 (21.3 vs. 9.1%) and a lower level of 18:2 (27.3 vs 35.9%) than reported by Comte et al. [24] for porcine heart.

TABLE V

Fatty acid composition of cardiolipin

Cardiolipin was extracted and purified and its fatty acid composition determined as described under Methods. Values for corpus luteum represent the mean of duplicate determinations from three experiments. Values for heart and liver represent the mean of duplicate determinations from a single experiment. In all cases the range was less than 5% of the mean.

Fatty acid	Composition, percent by weight		
	corpus luteum	heart	liver
14:0	0.00	0.00	1.38
15:0	0.00	0.00	2.20
16:0	5.57	7.69	8.31
16:1(9)	0.24	1.61	3.63
18:0	1.42	2.94	7.86
18:1(9)	2.97	15.59	5.09
18:1(11)	14.37	0.00	6.00
18:2(9,12)	61.31	67.43	51.16
18:3(6,9,12)	0.00	0.00	1.07
18:3(9,12,15)	0.49	0.42	1.07
20:2(11,14)	10.90	1.35	1.47
20:3(11,14,17)	0.19	0.00	0.00
20:4(5,8,11,14)	0.36	0.00	0.00
20:5(5,8,11,14,17)	0.95	0.65	2.58
22:0	0.00	0.00	2.28
22:2(13,16)	0.00	0.00	0.53

Fatty acid composition of cardiolipin

Table V shows the fatty acid composition of cardiolipins from porcine corpus luteum, heart and liver inner mitochondrial membranes. The major fatty acid in all three tissues was 18:2 which is typical of cardiolipins from many sources [25]. The next major component was 18:1 comprising between 11.1% and 17.3%. Interestingly two isomers of 18:1 were observed, namely oleic acid ($n-9$) and *cis*-vaccenic acid ($n-7$). The ratios of the two isomers varied between the tissues with corpus luteum containing predominately *cis*-vaccenic, heart predominately oleic and liver approximately equal proportions of both isomers. Another interesting finding was the high proportion of 20:2 in cardiolipin from corpus luteum but not heart or liver. Cardiolipins from all three tissues were highly unsaturated which is characteristic of this phospholipid and a good indicator of the purity of cardiolipin preparations [26].

Data presented here on the composition of cardiolipin in the porcine corpus luteum are very similar to those reported previously by Tuckey and Stevenson for the bovine corpus luteum [15]. The one exception is the bond configuration attributed to acid 18:1. The GLC system used then did not distinguish between the isomers of 18:1. Tuckey and Stevenson also reported higher levels of 20:4 (5.4% vs. 0.36%). Our values for porcine heart are similar to those reported by Comte et al. [24] and values for porcine liver are similar to values reported for rat liver inner membranes by Colbeau et al. [27].

Discussion

In any fractionation procedure the relative purity of the fractions greatly influences the results obtained. Since we were concerned with the inner membrane fraction, we looked specifically for contamination from other cellular membranes. Results with 5′-nucleotidase showed minimal contamination (2.9%) from plasma membranes. Outer membrane contamination was harder to determine due to the uncertainty of the location of alkaline phosphatase. Although only 1% of the activity was recovered in the inner membrane fraction, con-

tamination by outer membranes could have been as high as 8%. Contamination by endoplasmic reticulum could not be determined since luteal tissue does not contain glucose-6-phosphatase and we have been unable to devise a satisfactory alternative marker. However, due to the presence of alkaline phosphatase in the endoplasmic reticulum, any contamination from this fraction would have been detected along with outer membrane contamination. Overall the inner membrane fraction prepared here was relative pure and the levels of contamination should have had minimal effects on the results.

Quantitation of respiratory cytochromes in luteal tissues is complicated by the presence of cytochrome $P\text{-}450_{scc}$ since the α peak of its reduced minus oxidised spectrum overlaps the α peaks of both cytochrome *b* and cytochrome *c* [22]. This makes comparisons with non cytochrome *P*-450 containing tissues, such as heart, difficult. Nevertheless comparison of the relative values with unfractionated mitochondria demonstrates that the subfractionation procedure does not result in any selective removal or enhancement of these integral membrane proteins. Some loss of cytochrome-*c* was observed. Since this cytochrome is only loosely attached to the surface of the membrane, this result is not surprising. Nevertheless relative to haem *a*, total recovered cytochrome-*c* was higher than in heart.

The ratio of cytochrome $P\text{-}450_{scc}$ to haem *a* was 2.1 in luteal inner membranes giving a value of four moles of cytochrome $P\text{-}450_{scc}$ for each mole of cytochrome-*c* oxidase. Using values of 49 300 for the molecular weight of cytochrome $P\text{-}450_{scc}$ [28] and 140 000 for cytochrome-*c* oxidase [2], these two cytochromes represented 3.6% and 2.4%, respectively, of the inner membrane protein.

Cytochrome $P\text{-}450_{scc}$ requires a pool of inner membrane cholesterol as substrate. The size of this pool depends on both its rate of utilization for pregnenolone synthesis and the rate of input from outer membrane and the lipid droplet stores in the cytoplasm. In porcine corpus luteum the rate of pregnenolone synthesis from endogenous cholesterol (in vitro) is biphasic [16]. The initial rapid phase is proposed to reflect metabolism of cholesterol in a steroidogenic pool in the inner membrane, while the slow phase represents the rate of movement of cholesterol into that pool. Consequently, mitochondria actively synthesising pregnenolone after hormonal stimulation would have lower inner membrane cholesterol levels than non-stimulated mitochondria. The variation in cholesterol content we observed can therefore be explained in terms of the different hormonal status (resulting from differences in the maturity, estrous, diet and housing conditions) of the animals delivered to the abattoir on a particular day.

We were interested to know if the presence of cytochrome $P\text{-}450_{scc}$ at a 4-fold molar excess over cytochrome-*c* oxidase and the presence of cholesterol at up to 0.15 mol/mol phospholipid required an expansion of the phospholipid bilayer. In corpus luteum the phospholipid concentration relative to haem *a* was twice that of heart. This may indicate that such an expansion occurs.

Fatty acyl-CoAs are the major energy source of luteal tissue for both ATP and steroid hormone synthesis. These substrates donate electrons to FAD-linked dehydrogenases. As the respiratory and steroidogenic electron transport chains are kinetically linked then ubiquinone, as the electron acceptor for these dehydrogenases, could be involved. Such an involvement could imply increased ubiquinone levels in luteal inner membranes. The results here, however, show no difference in the concentration of ubiquinone (relative to haem *a*) between corpus luteum and heart. This does not necessary preclude such a role for ubiquinone.

Cardiolipin is essential for the activity of cytochrome-*c* oxidase [29] and appears to activate other components of the respiratory electron transport chain [1,25]. Cardiolipin also enhances the activity of cytochrome $P\text{-}450_{scc}$ by decreasing the K_m of the enzyme for cholesterol [15,30]. It is likely that the cardiolipin content of the inner membrane of luteal tissue is an important determinant of the rate of pregnenolone synthesis in vivo. The cardiolipin concentration relative to haem *a* was 50% higher in corpus luteum than heart in keeping with its extra role as an activator of cytochrome $P\text{-}450_{scc}$. Cardiolipin was present at a level that would enhance cholesterol side chain cleavage under subsaturating cholesterol conditions [31]. Such conditions are present in porcine corpus luteum [16 and this paper].

The fatty acid composition of the phospholipid of the inner mitochondrial membrane can be influenced by factors such as diet, age and temperature [1]. By comparing results from corpus luteum with heart and liver from pigs from the same source, we hoped that these effects would be minimized. It was notable that the fatty acid composition of cardiolipin differed between tissues. There was a high luteal level of acid 20:2, linoleic acid elongated by two carbons. It has been noted previously that esterified fatty acids in lipid granules in pig [32] and rat [33] ovary become longer and more unsaturated as development progresses. If cardiolipin undergoes a cycle of deacylation and reacylation as has been demonstrated for other phospholipids [34], it is reasonable to expect that its composition may change to reflect the increasing fatty acid chain length. The different proportions of the $(n-9)$ and $(n-7)$ isomers of octadecenoate are harder to explain. They may reflect tissue differences in the activities of the elongating and desaturating enzymes, since both isomers can be produced from palmitate depending on whether elongation or desaturation occurs first. Alternatively it may reflect a specific difference in incorporation of fatty acids into cardiolipin between the tissues.

The inner mitochondrial membrane of corpus luteum is different from non-steroidogenic tissues due to the presence of a second terminal oxidase, cytochrome *P*-450_{scc}. There appears to be an expansion of the phospholipid bilayer relative to cytochrome-*c* oxidase. This may be to accommodate cytochrome *P*-450_{scc} and its substrate, cholesterol. There is also an increase, relative to haem *a* in the content of cardiolipin, the phospholipid which activates cytochrome *P*-450_{scc}.

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