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ELECTRON PARAMAGNETIC RESONANCE STUDIES OF CYTOCHROME *P*-450 AND ADRENAL FERREDOXIN IN SINGLE WHOLE RAT ADRENAL GLANDS

EFFECT OF CORTICOTROPIN

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SUMMARY

Low and high spin ferric cytochrome *P*-450 and reduced adrenal ferredoxin (adrenodoxin) have been directly studied by EPR techniques in whole rat adrenal glands. The spectra obtained correspond closely to those obtained from sub-cellular fractions except in the case of low spin ferric cytochrome *P*-450, where there are differences in the shape of the $g = 2.41$ line. The relative magnitudes of these peaks in anaerobic and aerobic rapidly frozen adrenals from control and corticotropin stimulated hypophysectomised rats were used to investigate the control and rate limiting steps in adrenal steroid biosynthesis via cytochrome *P*-450. All adrenals showed a close to maximal level of reduced adrenodoxin and aerobic and anaerobic glands from control rats and aerobic glands from corticotropin stimulated rats showed similar quantities of low spin ferric cytochrome *P*-450. On anaerobiosis the quantity of low spin ferric cytochrome in adrenals from corticotropin stimulated rats dropped to 30–40 % of the aerobic level. Treatment of the rats with cycloheximide prior to administration of corticotropin prevented these changes. Approximately 0.4 % of the total cytochrome *P*-450 was high spin ferric in control adrenals and in aerobic stimulated adrenals this rose to approximately 0.6 %. These results demonstrate that association of substrate with cytochrome *P*-450 is the rate limiting step in adrenal steroidogenesis via cytochrome *P*-450. It is suggested on the basis of these and mitochondrial optical and EPR experiments that the limiting step being observed is cholesterol binding to cholesterol side chain cleavage cytochrome *P*-450, and that the rate of this association is stimulated by corticotropin.

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INTRODUCTION

One of the useful features of electron paramagnetic resonance (EPR) spectroscopy is the ability to make measurements on paramagnetic materials in intact whole tissues. Many metalloenzymes contain paramagnetic centres and as these often undergo oxidation and/or spin state changes during their functional cycles the potential exists to study dynamic processes in intact tissues, as was shown in a study of iron sulphur proteins in pigeon heart [1]. The need to make measurements at low temperatures does however introduce a number of technical complications in the freezing and handling of tissues. We have found the steroid synthesising cytochrome *P*-450 system in the rat adrenal most convenient to study in this way since cytochrome *P*-450 itself undergoes oxidation and spin state changes, and since adrenodoxin is EPR active only when reduced. The convenient size of the adrenal glands in 100–200 g rats avoids many problems of work up, as they can be directly admitted into standard (3 mm) EPR tubes. Furthermore a sufficiently good understanding of the behaviour of cytochrome *P*-450 in isolated adrenal mitochondria and microsomes (see ref. 3 and references cited) has been reached that the interpretation of results from whole glands can be attempted in a rigorous fashion.

The zona fasciculata-reticularis of the rat adrenal contains about 95% of the total cytochrome *P*-450 in the gland, whereas the zona glomerulosa contains about 5% [2]. Zona fasciculata-reticularis mitochondria contain about 80% of the total cytochrome *P*-450 whereas the microsomes contain about 15% [3]. Consequently the cytochrome *P*-450 in the zona fasciculate-reticularis mitochondria dominates the EPR spectra of the whole gland. Mitochondrial cytochrome *P*-450 is involved in cholesterol side chain cleavage ($P\text{-}450_{scc}$), 11β-hydroxylation ($P\text{-}450_{11\beta}$) and 18-hydroxylation. The microsomal enzyme is involved in 21-hydroxylation, and probably also reactions concerned with androgen formation. Cholesterol side chain cleavage is the first step in the conversion of cholesterol to steroid hormones and in the adrenal it is this step that is acutely activated by corticotropin [4]. The activation of steroidogenesis by corticotropin is inhibited by cycloheximide with a very rapid half-life for the decay of about 2–4 min [5]. The operative cycle of cytochrome *P*-450 as determined by optical and EPR studies of the enzyme in adrenal mitochondria and microsomes, liver microsomes and after isolation from bacterial sources is illustrated for the enzyme in rat adrenal mitochrondria in Fig. 1. In the absence of steroid substrates the heme iron is low spin ferric and gives an EPR spectrum with apparent g values of 2.414, 2.249 and 1.917. On binding substrate the heme iron becomes high spin ferric and we obtain apparent g_x values of 8.10 ± 0.02 for $P\text{-}450_{scc}$ bound to cholesterol and 7.80 ± 0.02 for $P\text{-}450_{11\beta}$ bound to deoxycorticosterone [3, 6, 7]. This spin state change on binding substrate corresponds to the 'type 1' optical change observed on adding appropriate substrates to *P*-450 preparations, a shift of $\lambda\ E_{max}$ Soret from 420 to 385 nm. In further steps in the cytochrome *P*-450 operative cycle the heme iron has no EPR spectrum. Low spin ferrous heme iron is diamagnetic and when high spin ($S = 4$) the energy separation of the levels between which transitions are to be observed may be too great for EPR spectra to be obtained at X-band (9 GHz) since the prediction of Kramer's [8] that there will be degeneracy of the appropriate energy levels in the absence of an externally applied magnetic field only applies to systems containing odd numbers of unpaired

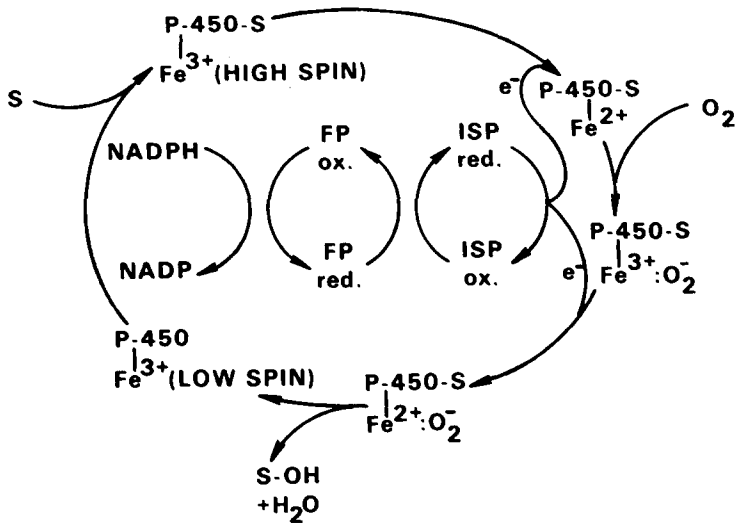

Fig. 1. Operative cycle for cytochrome *P*-450 in rat adrenal mitochondria projected from experiments with adrenal mitochondria, liver microsomes and isolated bacterial cytochrome *P*-450.

electrons. The proximal source of reducing equivalents for the cytochrome *P*-450 is a binuclear (two iron, two labile sulfur atoms) iron sulfur protein, adrenodoxin. Like other iron sulfur proteins of this type it has an EPR spectrum only after one electron reduction, two formally high spin iron atoms being antiferromagnetically coupled to give a net spin of 0 when oxidised, $\frac{1}{2}$ when reduced [9, 10]. We obtain g values of 2.02, 1.938 for the reduced protein.

The object of the present investigation was to examine the EPR spectra of cytochrome *P*-450 in the intact rat adrenal gland, and to study the effect of corticotropin on these spectra.

METHODS AND MATERIALS

Hypophysectomised rats, 150 g, female, 24 p.o. were obtained from Carworth U.K. Ltd. They were anaesthetised with ether and corticotropin (8 I.U. in 0.2 ml 0.001 M acetate, pH 4.0) was injected into the posterior vena cava. Control animals received an injection of acetate buffer alone. After 4 min the left adrenal was mobilised by cutting away the surrounding fat and a minute later the gland was inserted into a 3 mm EPR tube, the blood supply was cut off, and the tube was immediately immersed in liquid nitrogen. The time interval between the interruption of its blood supply and the gland being frozen was $\leqslant$ 10 seconds. The right adrenal was similarly removed and left to stand for varying times before being frozen in liquid nitrogen. Blood samples were taken before and five minutes after corticotropin or vehicle injection and plasma corticosterone levels determined by the method of Glick et al. [11]. EPR measurements were made at 77 K (low spin ferric cytochrome *P*-450, reduced adrenodoxin) in a liquid nitrogen finger dewar and at 9.0 K (high spin ferric cytochrome *P*-450) in an Oxford Instruments liquid helium cryostat as described in the accompanying paper [3]. Glands were also frozen with copper pliars which

had been precooled in liquid nitrogen [12]. For measurements at the lower temperatures it was necessary to crush samples frozen with the cooled pliars and to seal them in an evacuated EPR tube, both steps being carried out at approximately 77 K. The total cytochrome *P*-450 content of the glands was determined optically by the method of Omura and Sato [13], after homogenisation in 0.25 M sucrose, using an Aminco DW2 spectrophotometer. Cycloheximide was obtained from Nutritional Biochemicals Corporation.

RESULTS

Plasma corticosterone levels

Table I shows the plasma corticosterone levels before and 5 min after injection of either corticotropin or vehicle. It can be seen that there was a significant increase in the plasma corticosterone level at this time after corticotropin injection. Only animals which showed such an corticotropin-induced increase were included in this study.

The spectra

Fig. 2 illustrates a typical 77 K high field EPR spectrum of a whole adrenal gland frozen as described above. At $g = 2.414$, 2.249 are two of the three peaks of low spin ferric cytochrome *P*-450 (the third is obscured by the large adrenodoxin signal). There is a small but real difference in the shape of the $g = 2.414$ peak when compared with mitochondrial spectra [3]. It has not proved possible to simulate this lineshape by superposition of various mitochondrial and microsomal spectra. The height of the $g = 2.249$ line was used for quantitation of the level of low spin ferric cytochrome *P*-450. There were no measurable changes in lineshape at $g = 2.249$ when the intensity of this peak was halved on anaerobiosis. The peak to trough widths were 2.31 ± 0.02* mT for aerobic samples (8 determinations) and 2.32 ± 0.06* mT for anaerobic samples (8 determinations) where the average peak height of the anaerobic samples was 52 % of the aerobic value.

The 9.0 K spectra of whole adrenals showed signals close to noise level at

TABLE I

PLASMA CORTICOSTERONE LEVELS IN HYPOPHYSECTOMISED RATS

Experimental conditions		Plasma corticosterone level[a] (μg/100 ml)	
Control	before injection	2.68 ± 0.48 (12)	N.S.
	5 min after injection	4.03 ± 1.07 (12)	
Corticotropin	before injection	2.39 ± 0.62 (10)	$P < 0.0025$
	5 min after injection	9.71 ± 1.43 (10)	

[a] Results are presented as mean $\pm$S.E.M. (no. of observations). *P* values shown are for paired "*t*" tests.
N.S., not significant.

* Mean $\pm$S.E.M.

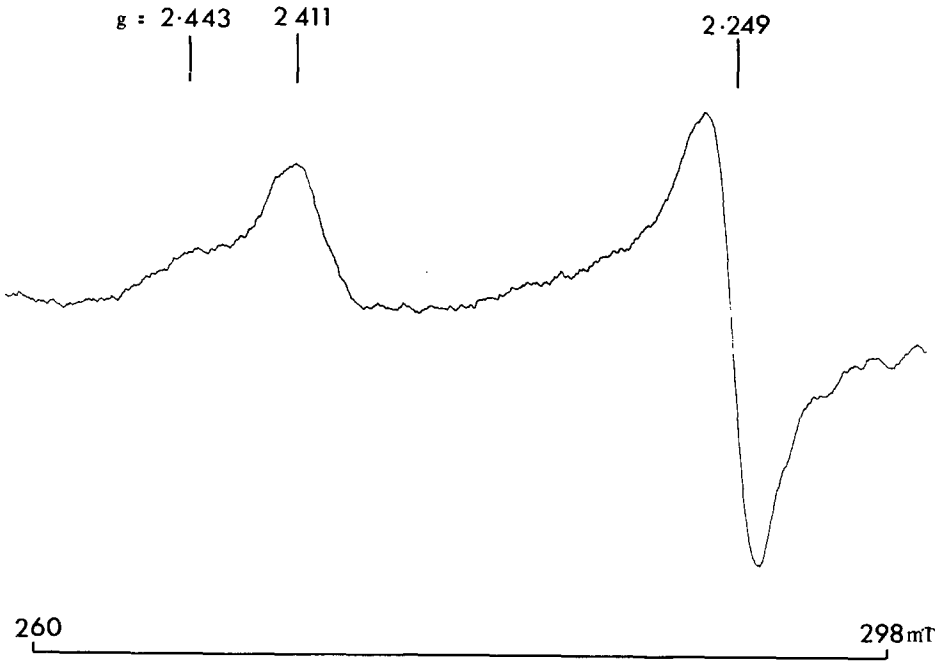

Fig. 2. Low field EPR line of low spin ferric cytochrome *P*-450 in a single whole rat adrenal (female, 150 g). Temperature 77 K, microwave frequency = 9.165 GHz.

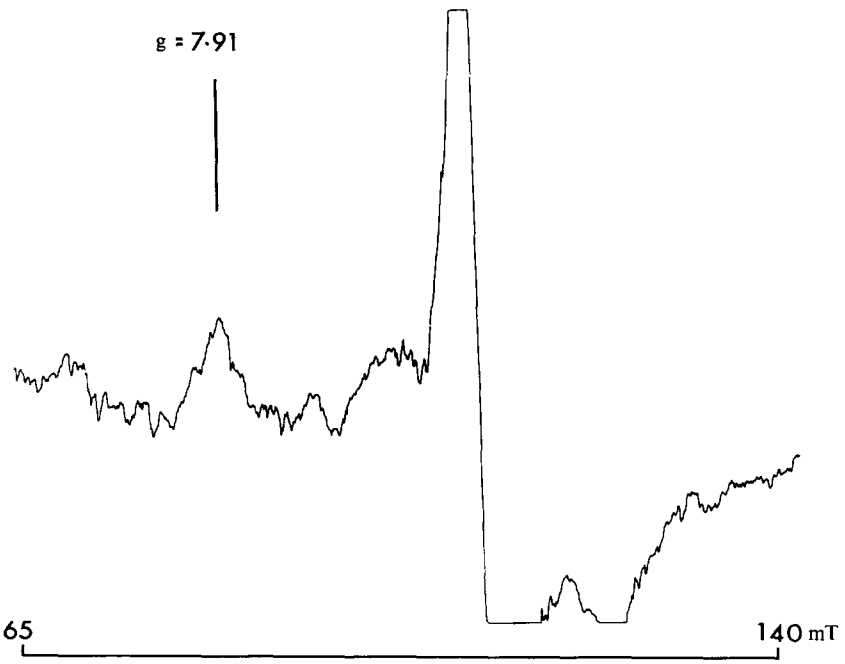

Fig. 3. EPR spectrum at low field and at low temperature (9.0 K) of a single whole rat adrenal gland (female, 150 g). Microwave frequency = 9.150 GHz.

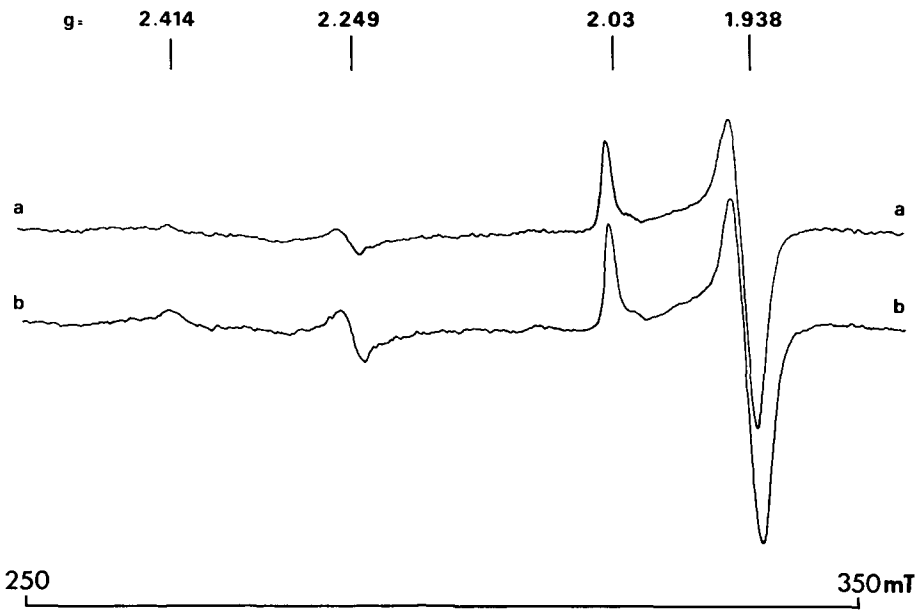

Fig. 4. EPR spectra of single whole rat adrenals (female, 150 g) at 77 K showing low spin ferric cytochrome *P*-450 ($g = 2.414, 2.249$) and adrenal ferredoxin ($g = 2.02, 1.938$). (a) frozen 3 min after resection; (b) rapidly frozen < 10 s after resection. Identical instrument settings were used for each spectrum. Microwave frequency = 9.172 GHz.

$g \approx 8$ under even the most favourable instrument settings. The most distinct of the 125 samples studied is shown in Fig. 3. The high spin ferric cytochrome *P*-450 has a g_x value of 7.90 corresponding more nearly to high spin microsomal *P*-450 or perhaps *P*-$450_{11\beta}$ rather than *P*-450_{scc} [3]. Statistical analysis of the weak peaks in the $g = 7.8$ region did however reveal changes in intensity between corticotropin stimulated and unstimulated rats, but it was not always possible to accurately assign *g*-values to these signals.

Fig. 4 shows typical whole gland EPR spectra run at 77 K over a wider range of magnetic field strength and lower sensitivity than the spectrum in Fig. 2. The two lines at $g = 2.02, 1.938$ correspond to reduced adrenodoxin, since identical *g* values were obtained from adrenodoxin in adrenal mitochrondria reduced with dithionite, isocitrate and succinate, and from a sample of reduced isolated bovine adrenodoxin (kindly supplied by Dr. R. Cammack, King's College). Although other iron sulphur proteins are present in mitochondria their contribution to the EPR spectra in the adrenal will be negligible, since components of the hydroxylation chain are present at about 10 times the concentration of respiratory chain components in adrenal mitochondria [14]. Furthermore we have found that the magnitude of the $g = 1.94$ signal in adrenal glands is 40 times greater than that in anaerobic whole liver on a wet weight basis, where signals from this type of iron sulphur protein are confined to the respiratory chain. The height at $g = 1.938$ was used to quantitate the level of reduced adrenodoxin.

The effect of corticotropin

Table II lists a comparison of the signal intensities obtained at 77 K of low spin ferric cytochrome *P*-450 and reduced adrenodoxin from control and corticotropin treated hypophysectomised rats when the adrenals were rapidly frozen (aerobic) and allowed to stand for 3 min at 20–25 °C before freezing (anaerobic). Fig. 4 shows typical spectra. Under all conditions the level of reduced adrenodoxin was unchanged. The quantity of cytochrome *P*-450 present in low spin ferric form was unchanged in aerobic and anaerobic control adrenals and in aearobic corticotropin-stimulated adrenals but on anaerobiosis the level dropped in corticotropin-stimulated adrenals. After five minutes at 20–25 °C a minimal level of 30-40 % of the aerobic peak height was reached and no further change took place; at 37 °C the same process was complete in 1 min (Fig. 5). When 10 mg of cycloheximide in 0.4 ml acetate (0.001 M, pH 3.5) was injected into the posterior vena cava one minute prior to administration of corticotropin this effect was not seen; no change in intensity at $g = 2.249$ could be detected on anaerobiosis in the presence of cycloheximide (Table II).

Intensity measurements were made at $g = 8.1$, 7.9 and 7.8 at 9.0 K as shown in Table III. The signals were quantitated approximately using data obtained from experiments where the pH of adrenal mitochondria was varied [3]. As the pH is lowered, increases at $g = 8.10$ correspond to decreases in intensity at $g = 2.249$. The intensity at $g = 7.9$ in aerobic corticotropin stimulated adrenals was only 0.6 % of the intensity increase at $g = 8.10$ in mitochondria for a drop in magnitude of the low spin signal equal to the total low spin signal found in control adrenal glands. In control adrenals this figure was 0.4 %. We consider that the low spin signal in control rats represents close to the total amount of cytochrome *P*-450 present in these glands, for reasons outlined in the discussion. The method of quantitation used here is only approximate at $g = 7.9$ since the standard for comparison has a different lineshape. Comparisons of whole gland signals with the magnitude of high spin signals at $g = 7.8$ obtained in adrenal mitochondria on addition of deoxy-

TABLE II

CONTENT OF FERRIC LOW SPIN CYTOCHROME *P*-450 AND REDUCED ADRENODOXIN IN SINGLE WHOLE RAT ADRENAL GLANDS

Experimental conditions		Ferric low spin *P*-450[a] (arbitrary units/nmol *P*-450)	Reduced adrenodoxin[a] (arbitrary units/nmol *P*-450)
Control	aerobic	4.70±0.32 (7)	25.83±1.28 (7)
	anaerobic	4.78±0.14 (7)	26.67±1.17 (7)
Corticotropin	aerobic	4.44±0.27 (6)	27.47±2.19 (6)
	anaerobic	2.50±0.20 (5)	25.42±1.61 (5)
Control	aerobic	4.83±0.14 (3)	25.60±0.60 (3)
+cycloheximide	anaerobic	4.41 (2)	25.4 (2)
Corticotropin	aerobic	4.81±0.37 (4)	27.20±2.00 (4)
+cycloheximide	anaerobic	5.09±0.30 (4)	30.80±0.90 (3)

[a] Mean ±S.E.M. (No. of experiments). Using unpaired "*t*" tests, the only groups to show a significant difference comparing control versus corticotropin, are corticotropin anaerobic versus control anaerobic for ferric low spin *P*-450, with $P < 0.005$.

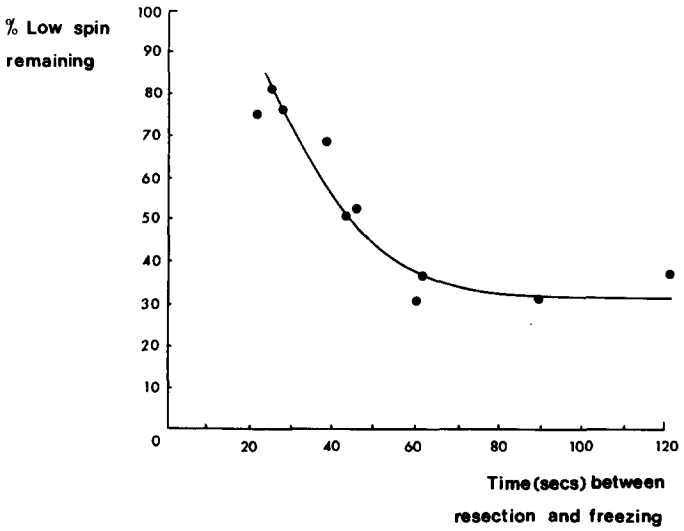

Fig. 5. Time course of disappearance of low spin ferric cytochrome *P*-450 in single whole adrenal glands from corticotropin injected female rats. The glands were removed from the animals and allowed to stand at 37 °C for the time interval indicated, prior to freezing in liquid nitrogen.

TABLE III

CONTENT OF FERRIC HIGH SPIN CYTOCHROME *P*-450 IN SINGLE WHOLE RAT ADRENAL GLANDS

Experimental conditions		Ferric high spin *P*-450[a] (arbitrary units/nmol *P*-450)		
		$g = 8.10$	$g = 7.90$	$g = 7.78$
Control	aerobic	0.849 ±0.516 (11)	1.316±0.806 (11)	0.677±0.292 (11)
	anaerobic	0.253 ±0.311 (12)	0.577±0.302 (12)	1.232±0.573 (12)
Corticotropin	aerobic	−0.0526±0.267 (9)	1.770±0.659 (9)	1.698±0.525 (9)
	anaerobic	0.469 ±0.401 (8)	1.194±0.821 (8)	1.563±0.626 (8)

[a] Results are presented as mean ±S.E.M. (No. of observations). Using unpaired "*t*" tests, the only groups to show a significant difference comparing control versus corticotropin, are corticotropin aerobic versus control aerobic at $g = 7.78$, with $P < 0.05$.

corticosterone gave good agreement (±20 %) with quantitation using the magnitude of the signals at $g = 8.1$. The relative changes at $g = 7.9$ are of course independent of the standard used. The data presented in Table III show that in aerobic glands, injection with corticotropin resulted in an increase in intensity at $g = 7.9$ and 7.8, but not at $g = 8.1$. However, only the increase at $g = 7.8$ was significant at $P < 0.05$.

The lineshape at $g = 2.414$ of low spin ferric cytochrome *P*-450 was compared in control and corticotropin treated rats. As a lineshape parameter the ratio of the heights at the field positions corresponding to $g = 2.442$, 2.414 was calculated and

from rapidly frozen stimulated glands a figure of 0.524±0.025 (16)* was obtained. After anaerobiosis the value was 0.625±0.033 (15)*.

DISCUSSION

The fact that in the intact adrenal gland from hypophysectomised rats there is no change in the level of reduced adrenodoxin whether the gland is rapidly frozen or anaerobic, from control or corticotropin-injected rats, suggests that under all these conditions the adrenodoxin is essentially fully reduced. Thus there is an adequate supply of reducing equivalents in the absence of corticotropin and under corticotropin stimulation. Consequently it is unlikely that corticotropin is acting to increase steroid biosynthesis by a mechanism involving increased supply of reducing equivalents.

In rapidly frozen corticotropin-stimulated adrenals the level of low spin ferric cytochrome *P*-450 is not detectably different from that in control aerobic and anaerobic adrenals and we would suggest that this represents close to the total heme iron in cytochrome *P*-450. The possibility that large amounts of substrate-free *P*-450 are reduced under physiological conditions is unlikely, because in perfused rat liver it is found that the heme iron in the hexobarbital metabolising cytochrome *P*-450 is only 6% ferrous in the absence of substrates [16], and in the camphor hydroxylating system of *P. putida* [17] it is observed that the redox potential of high spin cytochrome *P*-450 is higher than that of low spin *P*-450. It follows therefore that the cytochrome *P*-450 in rapidly frozen corticotropin-stimulated adrenals must be substantially in the substrate-free oxidised state, since the amount of low spin ferric *P*-450 is not significantly different from that in the control rats and the level of high spin ferric *P*-450 is very low, although apparently higher than in control adrenals. In anaerobic glands from corticotropin-stimulated rats, there is a substantial loss of low spin ferric *P*-450, but this does not happen in control anaerobic glands. There was no change in the total cytochrome *P*-450 content, as determined optically. On anaerobiosis the *P*-450 cycle must be blocked at the point of oxygen entry (Fig. 1) since reducing equivalents are demonstrated to be present and we would suggest that the low spin signal removed corresponds to an increase in substrate bound ferrous cytochrome *P*-450. Since there is no apparent change in the supply of reducing equivalents, we would suggest that the action of corticotropin has been to increase the amount of high spin ferric *P*-450, which is then available for reduction in the anaerobic situation.

These results therefore show that association of substrate with cytochrome *P*-450 is rate-limiting in the overall *P*-450 reaction cycle (Fig. 1) and it is this step which is activated by corticotropin. Furthermore it is this activation which is inhibited by cycloheximide. The observation of only very low levels of high spin (substrate bound) ferric cytochrome *P*-450 which are increased but remain low after corticotropin stimulation is consistent with this viewpoint. These conclusions are substantiated by previous optical and EPR results on isolated adrenal mitochondria [6, 18]. The cytochrome *P*-450 system in rat adrenals thus seems to contrast with the hexobarbital metabolising *P*-450 in perfused rat liver where on addition of up to 0.4 mM hexobar-

* Mean ±S.E.M. (no. of determinations).

bital to the perfusate spectral changes corresponding to substrate binding were observed, and up to 23 % of the cytochrome became reduced [19]. This figure was arrived at on the basis of optical changes occuring on perfusing with solutions containing 3.5 % (v/v) CO, which did not appear to affect oxygen uptake. Kinetic data from isolated camphor hydroxylase of *Pseudomonas putida* show that in this system, in the presence of excess substrate, reduction of the *P*-450 · substrate · O_2 complex by putidaredoxin is rate limiting [20].

The residual low spin *P*-450 signal which remains in anaerobic stimulated adrenals (a minimum of 30–40 % of the aerobic signal is obtained after extended periods of anaerobiosis at 37 °C) probably represents substrate-free forms of cytochrome *P*-450 responsible for 11β-hydroxylation, 18-hydroxylation and 21-hydroxylation since a large pool of cholesterol exists in these glands which we believe will continue to associate with cytochrome *P*-450_{scc} for a while after the onset of anaerobiosis. It is likely that the levels of substrates for these other forms of cytochrome *P*-450 would be relatively low in the stimulated state. It is interesting to note that on addition of cholesterol to acetone extracted bovine adrenal mitochondria absorbance differences ΔA (385–420 nm) of 0.735 were obtained on addition of cholesterol, and 0.605 on addition of deoxycorticosterone [21]. In a previous paper [3] we reported that deoxycorticosterone binding only accounted for some 20 percent of the mitochondrial cytochrome *P*-450 in rat adrenals as determined by EPR. Previous optical studies [22] suggested this number to be about 30 percent. Thus cholesterol-binding cytochrome *P*-450 may be the major *P*-450 component in this preparation.

We suggest therefore that the anaerobic change in the presence of corticotropin is due largely to the association of cholesterol with cytochrome *P*-450_{scc}. This is supported by recent data of Jefcoate and Orme-Johnson [23] on the steroid-binding spectra in mitochondria from rat adrenals subjected to varying degrees of anaerobiosis prior to homogenisation. Our interpretation of the anaerobic change is also supported by EPR measurements on mitochondria from anaerobic adrenals of control and corticotropin injected hypophysectomised rats. Here it was found that corticotropin increased the levels of substrate-bound *P*-450 at $g = 8.10$ (cholesterol bound) and $g = 7.80$ (deoxycorticosterone bound) but that the increase at $g = 8.10$ (cholesterol bound) was relatively larger (Fig. 6). Using the method for quantification previously described [3], we find an increase at $g = 8.10$ of 5.4 fold to 6.7 % of the total *P*-450 and at 7.80 of only 1.9 fold to 2.7 %. It is interesting that the proportion of the total *P*-450 in the high spin ferric form is greater in the isolated mitochondria than in the intact gland. During the preparation of the mitochondria, reducing equivalents are washed out as evidenced by the total absence of a reduced adrenodoxin EPR signal in the isolated mitochondria. Consequently it would be expected that during the preparation of the mitochondria, substrate-bound ferrous *P*-450 would be converted to substrate-bound ferric *P*-450. This explains the relatively greater proportion of *P*-450 in the high spin ferric form in the mitochondria as compared with whole glands and further substantiates the previous contention that the loss of low spin ferric *P*-450 in the corticotropin-stimulated anaerobic gland is due to the formation of substrate-bound ferrous *P*-450.

These EPR results, together with optical measurements on mitochondria from ether-stressed rats [18] indicate that the slow step in the conversion of cholesterol to steroid hormones is its association with mitochondrial cytochrome *P*-450

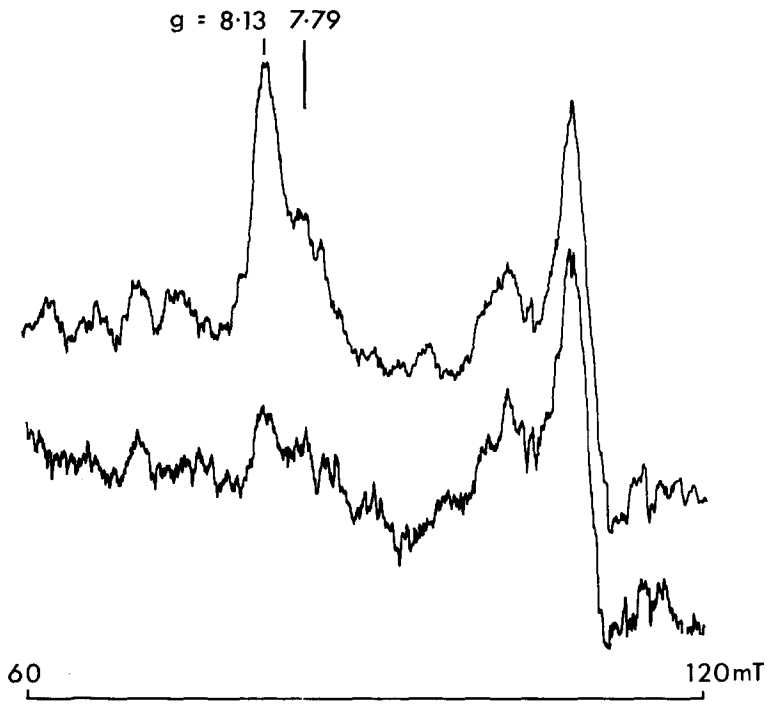

Fig. 6. EPR spectra of the low field line of high spin ferric cytochrome *P*-450 in adrenal mitochondria prepared from hypophysectomised rats (lower trace) and hypophysectomised rats which had been treated with corticotropin (upper trace). Identical instrument settings were used in each case. Temperature = 9.0 K. Microwave frequency = 9.146 GHz. Mitochondria were prepared as previously described [3] except that they were allowed to stand at room temperature for 5 min prior to insertion into EPR tubes and freezing in liquid nitrogen.

and this step is activated by corticotropin. Whether this activation represents an increase in the actual binding of cholesterol to cytochrome *P*-450 or an increase in intramitochondrial transport of cholesterol from another mitochondrial site, cannot yet be decided.

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REFERENCES

1 Orme-Johnson, N. R., Hansen, R. E. and Beinert, H. (1974) J. Biol. Chem. 249, 1928–1939
2 Simpson, E. R. and Williams-Smith, D. L. (1975) Biochim. Biophys. Acta 404, 309–320
3 Simpson, E. R. and Williams-Smith, D. L. (1976) Biochim. Biophys. Acta 449, 59–71

4 Stone, D. and Hechter, O. (1954) Arch. Biochem. Biophys. 51, 457–469
5 Schulster, D., Richardson, M. C. and Palfreyman, J. W. (1974) Mol. Cellular Endocrinol. 2, 17–29
6 Jefcoate, C. R., Simpson, E. R., Boyd, G. S., Brownie, A. C. and Orme-Johnson, W. H. (1973) Ann. N.Y. Acad. Sci. 212, 243–261
7 Brownie, A. C., Alfano, J., Jefcoate, C. R., Orme-Johnson, W. H., Beinert, H. and Simpson, E. R. (1973) Ann. N.Y. Acad. Sci. 212, 344–360
8 Kramers, H. A. (1930) Proc. Acad. Sci. Amst 33, 959
9 Gibson, J. F., Hall, D. O., Thornley, J. H. M. and Whatley, F. R. (1966) Proc. Natl. Acad. Sci., U.S.A. 56, 987–990
10 Blumberg, W. E. and Peisach, J. (1974) Arch. Biochem. Biophys. 162, 502–512
11 Glick, D., Von Redlich, D. and Levine, S. (1964) Endocrinology 74, 653–655
12 Wollenberger, A., Ristau, O. and Schoffa, G. (1960) Pflugers Arch. Ges. Physiol. Menschen 270, 339–412
13 Omura, T. and Sato, R. (1964) J. Biol. Chem. 239, 2379–2385
14 Cammer, W., Cooper, D. Y. and Estabrook, R. W. (1967) in Functions of the Adrenal Cortex (McKerns, K., ed.), p. 943, Vol. 2, Appleton Century, Crofts
15 Mason, J. I., Estabrook, R. W. and Purvis, J. L. (1973) Ann. N.Y. Acad. Sci. 212, 406–419
16 Brauser, B., Versmold, H. and Bucher, Th. (1968) Hoppe Seylers Z. Physiol. Chem. 349, 1589
17 Peterson, J. A. (1971) Arch. Biochem. Biophys. 144, 678–693
18 Simpson, E. R., Jefcoate, C. R., Brownie, A. C. and Boyd, G. S. (1972) Eur. J. Biochem. 28, 442–450
19 Sies, H. and Brauser, B. (1970) Eur. J. Biochem. 15, 531–540
20 Tyson, C. A., Lipscomb, J. D. and Gunsalus, I. C. (1972) J. Biol. Chem. 247, 5777–5784
21 Cheng, S. C. and Harding, B. W. (1973) J. Biol. Chem. 248, 7263–7271
22 Jefcoate, C. R., Simpson, E. R. and Boyd, G. S. (1973) Eur. J. Biochem. 42, 539–551
23 Jefcoate, C. R. and Orme-Johnson, W. H. (1975) J. Biol. Chem. 250, 4671–4677