Oxygen consumption, carbon dioxide production and progestagen secretion in the intact ovary of the Day-16 pregnant rat*

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Summary. Arterial and venous blood gases were measured in the ovary of the Day-16-pregnant rat by a Van Slyke manometric technique. Concurrent observations of progestagen concentrations were also made to determine rates of hormone secretion. The oxygen consumption was 196·5 ± 28·4 ml/min per kg ovarian tissue (mean ± s.e.m., n = 8) which is amongst the highest recorded from any organ. Carbon dioxide production was 149·8 ± 36·6 ml/min per kg ovarian tissue (n = 5) and the respiratory quotient was 0·756 ± 0·023 (n = 5), indicating that lipids are the major energy substrate used by the ovary. The rates of progestagen secretion were 2·12 ± 0·37 and 0·42 ± 0·10 nmol/min per ovary for progesterone and 20α-dihydroprogesterone, respectively, and were not related to oxygen consumption. Less than 1·5% of the oxygen consumed was used in the essential conversion of cholesterol to pregnenolone, the immediate precursor of progesterone.

Introduction

The basic metabolism of the corpus luteum (CL) and its relation to steroidogenesis has received little attention. In-vitro studies suggest that luteal cells have a high rate of metabolism with an oxygen consumption equivalent to 3 ml/min per kg of ovarian tissue (Flint & Denton, 1969). From glucose utilization studies (Armstrong, 1968; Flint & Denton, 1970) and estimated respiratory quotients of homogenized CL tissue (Stevenson et al., 1973), fatty acids are thought to be a major energy substrate. The relevance of these findings to the in-vivo situation, however, is uncertain. Oxygen consumption is much higher in auto-transplanted ovaries of sheep (40 ml/min per kg ovary; Baird et al., 1973) and intact human ovaries (14 ml/min per kg ovary; Fraser et al., 1973). However, oxygen consumption has not been correlated with progesterone secretion and there is as yet no information on carbon dioxide production, respiratory quotients or energy substrate utilization in the intact ovary.

In the present work oxygen consumption, carbon dioxide evolution and progestagen secretion were monitored simultaneously from the intact ovary of rats at Day 16 of gestation, a time of maximal progesterone secretion (Uchida et al., 1970). The data obtained were used to determine (1) whether variation in progestagen secretion was related to oxygen consumption, (2) the proportion of oxygen consumed that was directly involved in the conversion of cholesterol to pregnenolone, and (3) the respiratory quotient of ovarian tissue as a guide to the energy substrate used.

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Materials and Methods

Nulliparous albino Wistar rats (Animal Resources Centre, Murdoch, W.A.) 3-5 months old and weighing 211 ± 3.6 g (mean ± s.e.m., N = 8) at mating were used. Rats were kept in an environmentally controlled room (17-23°C, relative humidity 50-70%, lights on from 07:00 to 21:00 h). Food and water were freely available. Rats were mated during darkness and the morning on which spermatozoa were found in a vaginal smear was called Day 1 of gestation. Rats from this colony normally litter on the morning of Day 23.

Reagents. Reagents were analytical reagent grade and usually purchased from Ajax Chemicals, Sydney, Australia. Sodium hyposulphite was purchased from Sigma Chemical, St Louis, MO, U.S.A.; sodium anthroquinone-β-sulphonate and hydrogen peroxide from BDH Chemicals, Sydney, Australia.

Blood gas determinations. The method was based on a micro-version of the Van Slyke manometric technique as described by Van Slyke & Plazin (1961) using refinements suggested by Bartels et al. (1963). The apparatus, patterned on that described by Bartels et al. (1963) was constructed by University Glassblowers, University of Western Australia, with the exception of the extraction chamber (Cat. no. 9695 F80; W. Thomas, Philadelphia, PA, U.S.A.).

To make a determination, a 90-µl sample of blood was taken anaerobically from a cannulated blood vessel (see below) into a Hamilton syringe (250 µl, SGE, Ringwood, Victoria, Australia) previously filled with 10 µl NaF (10%, w/v). The sample was injected directly into the extraction chamber of the manometric apparatus or kept on ice for no longer than 20 min then added to the chamber. Once added to the chamber, the blood and the NaF were mixed with the extraction solution (0.7 ml of a mixture of lactic acid (5% v/v), potassium ferricyanide (16 g/l), saponin (4 g/l) and octyl alcohol (2.25 ml/l)) using a magnetic stirrer and the pressure in the chamber was reduced to about -760 mmHg. The solution was stirred for 5 min to extract the gases (oxygen, carbon dioxide and nitrogen), then the volume of gas evolved was compressed to 0.1 ml and the pressure required was recorded (P1). This was normally repeated 3 times or until constant P1. Potassium hydroxide (1 ml 5 M) was added to the chamber to absorb the carbon dioxide, the solution was mixed, the gases were recompressed to standard volume (0.1 ml) and the pressure was recorded (P2). To absorb oxygen, 0.1 ml of a final solution (sodium dithionite, 200 g/litre KOH (10 M) with anthroquinone-β-sulphonate (20 g/l) was added and the pressure (P3) was taken. The oxygen content of the blood was calculated according to the formula:

\[ O_2 = ((P2 - P3 - c) \times f) \times (100/90) \]

where P2 and P3 are pressure recordings, c is the blank (reagents only), recording, f is a chamber/volume factor with allowance for room temperature, and (100/90) is a correction for the dilution of the sample with NaF. The carbon dioxide content was calculated similarly:

\[ CO_2 = ((P1 - P2 - c) \times f) \times (100/90) \]

All estimates were calculated as mmol/l but were converted to ml/min per kg ovary for comparison with results of other workers.

Although the technique has been described as highly accurate, only limited data are available on precision and accuracy. The present work required a high degree of precision since differences between arterial and venous blood samples were expected to be small and respiratory quotients a function of differences in carbon dioxide divided by the differences in oxygen. It was calculated that the coefficient of variation of individual estimates would have to be < 1% to give a meaningful estimate for respiratory quotient. Initial experience with the technique suggested that this would be impractical and previous workers had referred to similar difficulties (Fraser et al., 1973). However, by using a calibrated magnifying glass (× 10) to read the mercury column pressure, inhibiting metabolism in stored samples with NaF and applying strict attention to cleaning the chamber thoroughly between measurements, the required precision, as calculated above, was obtained.

To validate the carbon dioxide measurements, two bicarbonate solutions, which covered the expected physiological range, were first measured volumetrically (Vogel, 1961) then with the Van Slyke apparatus. The volumetric determinations gave values of 13.04 ± 0.01 and 23.52 ± 0.08 mm (mean ± s.d., n = 6 and 9 respectively); the Van Slyke determinations gave values of 13.18 ± 0.04 and 23.56 ± 0.08 mm (n = 7). The methods gave a significantly different result (P < 0.01, unpaired t test) for the less concentrated solution only, and since the actual difference was only 1% it could be ignored safely. Furthermore, the coefficients of variation of repeated estimates on the Van Slyke apparatus were within the acceptable level of 1%.

To validate oxygen measurements, repeated estimates were made over a period of 6 h on blood from the carotid artery of a rat lightly anaesthetized with ether and from the vena cava of a rat, similarly anaesthetized but with severe hypoxia due to respiratory difficulties. The values obtained (11.33 ± 0.13, n = 7, and 0.403 ± 0.002, n = 5, respectively) confirmed that there was no apparent change over the 6-h period of measurement, and that the coefficient of variation was within the acceptable level of 1%. To test for linearity a series of blood samples covering the expected range of oxygen concentrations were measured with the Van Slyke apparatus and again with a Hemoximeter (Radiometer, Copenhagen, Denmark). The results from the Hemoximeter were converted to oxygen content (ml/100 ml blood) by multiplying oxygen saturation × haemoglobin content × 1.38 (Passmore & Robson, 1976a).
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and its linear regression on the Van Slyke values was determined. The regression coefficient was \(1.009 \pm 0.022\), the intercept on the Y axis was \(-0.383\) and the correlation coefficient was 0.9987, all of which clearly confirms the high level of accuracy and repeatability in both methods.

**Surgery and blood samples.** Rats were anaesthetized with an i.p. injection of pentobarbitone sodium (Nembutal: Abbott Laboratories, Sydney, Australia) and surgically prepared for ovarian venous outflow as previously described (Bruce & Meyer, 1981). The important features of the technique were that each rat was heparinized (500 i.u. in 0.5 ml saline), the ovarian venous drainage was completely isolated, the rat was positioned with its abdomen immersed in a saline bath at 37°C and the venous effluent was pumped back into the rat to maintain blood volume. Breathing rate, arterial blood pressure, temperature and heart rate were monitored throughout the experiment and arterial and venous blood samples were taken to analyse blood gases and to assess steroid secretion. Samples for steroid assay were stored at \(-10°C\) until assayed. At the end of the experiments both ovaries were removed and the corpora lutea of pregnancy were separated from those of the previous cycles as previously described (Swann & Bruce, 1986) and analysed for progestagen and total cholesterol content.

**Analysis.** Progesterone and 20α-dihydroprogesterone determinations were carried out by RIA as previously described (Swann & Bruce, 1986). Intra- and inter-assay coefficients of variation were 6.1% and 10.0% respectively for progesterone and 4.5% and 11.4% respectively for 20α-dihydroprogesterone.

Total cholesterol in tissue samples was determined by the cholesterol oxidase method (Sigma Chemical) as previously described (Swann & Bruce, 1986).

**Statistical analysis.** Student’s \(t\) tests, paired and unpaired as indicated, were usually applied. Correlations between steroid secretion and blood gas changes were assessed by analysis of covariance (Snedecor & Cochran, 1967). Values given are means \(\pm\) s.e.m. except when otherwise indicated.

**Results**

Eight rats were included in the results and within each rat 3 serial measurements were taken at 40-min intervals for blood gas analysis. Carbon dioxide values were not obtained from 3 rats due to apparatus failure and, in 1 rat, one oxygen measurement failed. The maternal weight gain (58.6 \(\pm\) 3.7 g) was similar to that previously observed in this colony of rats.

Comparison of the cannulated ovary to the contralateral ovary showed no differences in the weight of the various components (paired \(t\) test, see Table 1). Furthermore, examination of both ovaries from the experimental rat showed no significant differences in progesterone, 20α-dihydroprogesterone or total cholesterol content (see Table 1).

The preparation was stable over the 2-h measurement period in that ovarian blood flow, oxygen consumption, carbon dioxide production, respiratory quotient (RQ) and progesterone secretion

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**Table 1.** Data from rats at Day 16 of gestation subjected to ovarian venous outflow and blood gas measurements

<table>
<thead>
<tr>
<th></th>
<th>Cannulated side</th>
<th>Contralateral side</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of live fetuses</td>
<td>7.1 ± 0.6</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td>No. of CL</td>
<td>6.1 ± 0.5</td>
<td>6.0 ± 1.2</td>
</tr>
<tr>
<td>Ovary wt (mg)</td>
<td>64.1 ± 6.8</td>
<td>54.5 ± 3.4</td>
</tr>
<tr>
<td>Luteal wt (mg)</td>
<td>25.3 ± 4.0</td>
<td>20.2 ± 4.1</td>
</tr>
<tr>
<td>Stromal wt (mg)</td>
<td>38.8 ± 4.7</td>
<td>34.2 ± 2.0</td>
</tr>
<tr>
<td>Mean CL wt (mg)</td>
<td>4.09 ± 0.48</td>
<td>3.41 ± 0.23</td>
</tr>
<tr>
<td>Steroid conc. (nmol/g CL tissue)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>17.85 ± 1.30</td>
<td>16.68 ± 3.59</td>
</tr>
<tr>
<td>20α-Dihydroprogesterone</td>
<td>13.16 ± 2.62</td>
<td>10.58 ± 2.28</td>
</tr>
<tr>
<td>Cholesterol ((10^-3))</td>
<td>23.42 ± 7.61</td>
<td>30.65 ± 7.07</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) s.e.m., \(n = 8\); ovarian tissue weights were not recorded for 3 of the 8 rats used. There was no significant difference between sides (paired \(t\) tests).
Table 2. Comparison of data obtained at the first and last samples of the measurement period in rats subject to blood gas measurement at Day 16 of pregnancy

<table>
<thead>
<tr>
<th></th>
<th>First sample</th>
<th>Last sample</th>
<th>Average of all values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian blood flow (ml/min)</td>
<td>0.35 ± 0.03</td>
<td>0.42 ± 0.06</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>Progesterone secretion (nmol/min)</td>
<td>2.06 ± 0.33</td>
<td>2.07 ± 0.38</td>
<td>2.12 ± 0.37</td>
</tr>
<tr>
<td>20α-Dihydroprogesterone secretion (nmol/min)</td>
<td>0.35 ± 0.06</td>
<td>0.50 ± 0.11</td>
<td>0.42 ± 0.10</td>
</tr>
<tr>
<td>Oxygen consumption (µl/min)</td>
<td>10.5 ± 1.2</td>
<td>9.2 ± 2.1</td>
<td>11.6 ± 1.9</td>
</tr>
<tr>
<td>Carbon dioxide production (µl/min)</td>
<td>8.0 ± 1.1</td>
<td>6.4 ± 2.1</td>
<td>8.8 ± 1.4</td>
</tr>
<tr>
<td>RQ</td>
<td>0.757 ± 0.041</td>
<td>0.734 ± 0.064</td>
<td>0.756 ± 0.023</td>
</tr>
</tbody>
</table>

Values are means ± s.e.m., n = 8 except for the carbon dioxide, oxygen consumption and RQ values when n = 5.

There was no significant difference between the first and last values (paired t tests).

Table 3. Correlations between oxygen consumption, ovarian blood flow and steroid secretion, both within individual rats and between rats

<table>
<thead>
<tr>
<th></th>
<th>Correlation†</th>
<th>Correlation‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen consumption (ml/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone (nmol/min)</td>
<td>0.103</td>
<td>-0.030</td>
</tr>
<tr>
<td>20α-Dihydroprogesterone (nmol/min)</td>
<td>-0.023</td>
<td>0.495</td>
</tr>
<tr>
<td>Total progestagens (nmol/min)</td>
<td>0.246</td>
<td>0.662</td>
</tr>
<tr>
<td>Blood flow (ml/min)</td>
<td>0.294</td>
<td>0.812*</td>
</tr>
<tr>
<td>Ovarian blood flow (ml/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone (nmol/min)</td>
<td>-0.375</td>
<td>0.845**</td>
</tr>
<tr>
<td>20α-Dihydroprogesterone (nmol/min)</td>
<td>0.052</td>
<td>0.561</td>
</tr>
<tr>
<td>Total progestagens (nmol/min)</td>
<td>-0.308</td>
<td>0.817*</td>
</tr>
</tbody>
</table>

All values compared are expressed per unit mass of tissue.

Values are r values for linear regression, *P < 0.05, **P < 0.01.
†Common correlation coefficients, from analysis of covariance.
‡Simple linear correlations based on mean values for each rat.

Rates showed no significant changes (paired t test) between the first and last samples examined (see Table 2). Similarly, arterial blood pressure, heart rate and respiration rate showed no significant differences over the measurement period. The overall stability of the preparation was confirmed by two-way analysis of variance which revealed no significant variation between the three serial measurements.

Oxygen consumption calculated as the mean of all values obtained was 196.5 ± 28.4 ml/min per kg ovarian tissue (mean ± s.e.m., N = 8) and the carbon dioxide production measured in 5 rats only was 149.8 ± 36.6 ml/min per kg ovarian tissue.
The RQ was 0.756 ± 0.023 (mean ± s.e.m., N = 5) and this was calculated from the data from 5 rats in which both measurements were taken (oxygen consumption 192.7 ± 40.7 ml/min per kg ovary, mean ± s.e.m., N = 5).

Oxygen consumption, steroid secretion and ovarian blood flow data were examined in detail. Two basic questions were asked. First, are changes in oxygen consumption or rates of ovarian blood flow within each rat related to progesterone secretion rates? Second, is metabolic activity in different rats, as indexed by oxygen consumption and rate of ovarian blood flow per unit mass of CL tissue, related to progestagen secretion rate per unit mass of CL tissue? The first question was tested by analysis of covariance (ANCOVA). The second question was tested by applying linear regression to the mean value of each measure for individual rats.

There was no evidence that changes in oxygen consumption or rate of blood flow within individual rats were related to rates of either progesterone or 20α-dihydroprogesterone secretion. Since there were no significant changes within rats, it was valid to compare rat mean values by linear regression. There was some indication that rats whose CL received higher blood flow consumed more oxygen (r = 0.812) and had higher rates of progesterone (r = 0.845) and total progestagen (r = 0.817) secretion (see Table 3). No other significant relationships were observed. ovaries with a higher mass of CL tissue tended to have a higher blood flow, oxygen consumption and progesterone secretion rates (r = 0.322, 0.486 and 0.143 respectively) but these trends were not significant.

Discussion

The measurement of blood gas changes was performed in rats whose ovaries were functionally active as assessed by the rate of secretion of progestagens (Bruce & Meyer, 1981). The cannulation procedure had no apparent local or systemic effect in that progestagen and cholesterol concentrations were similar in the two ovaries at the end of the experiment and to ovaries not subjected to surgical preparation (Wiest, 1970).

Ovarian oxygen consumption has not previously been reported for the rat ovary in vivo. The present result of 197 ml/min per kg of ovary indicates a remarkably high level of metabolic activity and, as far as we know, is higher than that recorded for any other organ measured under comparable conditions including the brain, liver, kidney and heart (33, 44, 61 and 94 ml/min per kg tissue respectively; Diem & Lentner, 1970). It is also 5 and 14 times greater than that reported for the autotransplanted ovary of the sheep (Baird et al., 1973) and human ovary (Fraser et al., 1973) respectively, but this may reflect differences in rates of hormone secretion which were not reported in these last two studies. Comparison with in-vitro studies is also limited since the only oxygen consumption values available were derived from superovulated ovaries of the immature rat (3 ml/min kg ovary: Flint & Denton, 1969) and were less than 1.5% of that found for the intact ovary in this study. These differences in metabolic activity reflect steroid secretion rates which, in a variety of in-vitro techniques reach only 1–6% (Gafvels & Selstam, 1985) of the in-vivo situation (Hashimoto & Wiest, 1969; Uchida et al., 1970; Bruce & Meyer, 1981). In view of this gross disparity, however, it would be unwise to claim an oxygen consumption and progesterone secretion relationship using the comparison of in-vitro to in-vivo findings as the evidence.

Given that about 90% of the ovarian blood flow is directed to the CL at Day 16 of gestation (Bruce et al., 1984) it is reasonable to assume that a similar proportion of oxygen is also consumed by this tissue. The proportion of the total oxygen consumed that is directly utilized in steroidogenesis can then be estimated. Three molecules of oxygen are consumed in the synthesis of one molecule of pregnenolone from cholesterol (Shikita & Hall, 1974). Pregnenolone is required for both progesterone and 20α-dihydroprogesterone synthesis and so the amount of oxygen required equals three times the total progestagen secretion rate (2.5 nmol/min per ovary). This is equivalent to 2.8 ml/min per kg ovary which is less than 1.5% of the total oxygen consumed by the ovary. The conversion of cholesterol to pregnenolone therefore places an insignificant demand on the total supply of oxygen.
Oxygen consumption is related to energy production and the energy substrate used is indicated by the respiratory quotient (RQ) of the tissue (Passmore & Robson, 1976a). The RQ value of 0.756 observed here implies that lipid metabolism is the major energy source for the ovary of the rat. This accords with Flint & Denton's (1969) finding that there is very little utilization of glucose by the rat ovary in vitro. It agrees closely with RQ values reported for whole ovary homogenates (0.71; Stevenson et al., 1973) despite the substantial difference in metabolic activity of in-vitro and in-vivo preparations referred to earlier.

The high oxygen consumption rate, together with the RQ value of 0.756, indicates a substantial requirement for lipid as an energy precursor. One possible source is the ester moiety of the cholesterol esters taken up from plasma as lipoproteins (Strauss et al., 1982). Generally the lipid-protein uptake is considered in terms of its function in providing the cholesterol precursor for pregnenolone, although Tuckey & Stevenson (1979) showed that fatty acids are an important energy source for the ovary in vitro. The amount of fatty acid required for oxidation can be determined by assuming that they are fully oxidized and the significant fatty acids available range from C16 to C22 (Tuckey & Stevenson, 1979, 1980) and require 23–31 oxygen molecules per molecule of fatty acid. Since the oxygen consumption is 11.6 µl/min per ovary, or 517 nmol/min, then 17–22 nmol/min fatty acids are required. Even if all the cholesterol used for steroidogenesis is esterified, with a progestagen secretion of 2.54 nmol/min per ovary, only that amount is available for energy production or less than 15% of the amount estimated above. It would seem that either free fatty acids are obtained directly from the blood for energy utilization or that a high proportion of cholesterol esters taken up by the ovary are used for purposes other than steroidogenesis.

Within individual rats there was no indication of a relationship between oxygen consumption and progestagen secretion or oxygen consumption and blood flow. This might now be expected given that only 1.5% of the oxygen consumed was directly attributable to the major step of pregnenolone synthesis. Furthermore, even if some other aspect of steroid synthesis and secretion utilizes higher amounts of oxygen, there could be a delay between the incorporation of oxygen and the release of progestagens which would obscure any direct relationship given the 40-min serial sampling system used here. The finding that those rats which had higher rates of ovarian blood flow also had higher oxygen consumption and progestagen secretion rates points to differences in metabolic activity between rats but not to causal relationships between these measures.

The high oxygen consumption must be considered when interpreting the significance of the high rate of ovarian blood flow. If the flow rate was, for example, only 25% of that observed then, by extrapolation of oxygen dissociation curves (Passmore & Robson, 1976b), oxygen tension in the ovarian vein would have to fall to 40 mmHg to maintain the same oxygen consumption rate. This could be important if the maintenance of the redox state of steroidogenic tissue requires a high oxygen tension in the surrounding blood (Lubbers & Kessler, 1968). This high oxygen consumption also suggests a high energy consumption and, as a necessary by-product, heat production. This heat can be estimated by multiplying the oxygen consumed (11.6 µl/min per ovary) by 0.00469 (calories generated per µl of oxygen consumed, at RQ = 0.71; Passmore & Robson, 1976c) and assuming that 60% of the available energy is lost as heat. If this heat (0.032 cal) is fully dissipated into the bloodstream then, with a blood flow of 0.41 ml/min, the blood temperature would rise by 0.08°C. This temperature rise would scarcely warrant a high blood flow simply to act as a heat sink.

In conclusion, the major finding of the present work was the high oxygen consumption of the ovary. Although similar physiological studies have been carried out in the testis (Setchell & Waites, 1964), only a small proportion of the total tissue is steroid secreting and consequently the results have limited comparative value to the present work. Indeed, the oxygen consumed per kg testis (5.8 ml/min) is only 3% of the ovarian consumption. The question as to why the ovary has such a high rate of consumption is therefore still open. It is possible that, over and above cell maintenance, a significant proportion of the oxygen consumed and the energy generated is needed for transport of cholesterol or other precursors into the luteal cell, sequestration and transport of progestagens from the cell and perhaps the synthesis and secretion of other products including proteins (Willcox...
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& Bruce, 1983) and relaxin (Anderson & Sherwood, 1984). It may be easier to explain the very high oxygen consumption rate when the secretion rates and stoichiometry of these products are determined.

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References


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