The effect of human pregnancy on the pulmonary transfer factor for carbon monoxide as measured by the single-breath method

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Summary
1. The pulmonary transfer factor for carbon monoxide was measured by the single-breath method in 21 pregnant women with no previous history of cardiac or respiratory disease. Measurements were made at monthly intervals throughout pregnancy and once post partum.
2. The transfer factor was higher in the first trimester of pregnancy than in the non-pregnant state. There was a fall in the transfer factor during pregnancy until 26 weeks gestation, after which no further decrease was observed.
3. The changes in transfer factor were not explained by alterations in haemoglobin concentration or alveolar volume.
4. Simultaneous serial estimations of plasma 17β-oestradiol were performed in all the subjects. There was no obvious direct relation between changes in the concentration of this hormone and transfer factor measurements.

Key words: carbon monoxide, pregnancy, respiratory function, transfer factor.

Introduction
Dyspnoea occurs in at least 60% of normal subjects during pregnancy (Thomson & Cohen, 1938; Cugell, Frank, Gaensler & Badger, 1953), but there have been only limited studies of alterations in the physiology of the lung. The pulmonary transfer factor for carbon monoxide has previously been measured on only three occasions during pregnancy and only one study has compared such values with those measured in the non-pregnant state (Gazioglu, Kaltreider, Rosen & Yu, 1970). These previous studies have tended to suggest that the transfer factor is unaltered during pregnancy (Bedell & Adams, 1962; Krumholz, Echt & Ross, 1964; Gazioglu et al., 1970). However, it is misleading to draw conclusions from such studies on small numbers of subjects, at infrequent intervals during pregnancy, for by such methods known changes in other physiological variables, e.g. plasma osmolality, in pregnancy would not have been detected (Robertson, 1968).

We have therefore measured transfer factor serially throughout pregnancy in a sufficiently large number of normal subjects to define the nature of any changes, and also to take account of factors known to affect transfer factor, such as haemoglobin concentration and lung volume. We have also made serial measurements of 17β-oestradiol in all the subjects to investigate any relation between the levels of this hormone and the transfer factor values, as Pecora, Putnam & Baum (1963) found transfer factor to be reduced after intravenous oestrogens.

Subjects and methods
We studied 21 healthy pregnant women aged from 21 to 34 years. All were studied initially in the first trimester of pregnancy, when the average gestational age was 10 weeks. All investigations were repeated at monthly intervals...
thereafter. Each subject was studied once between 3 and 5 months after delivery. All patients gave their informed consent to the study, which had been approved by the institution's Ethical Committee.

None of the women gave a history of previous cardiorespiratory disease and no abnormality of this system was detected on physical examination. All had normal pregnancies and remained normotensive throughout. The cigarette consumption did not increase in any subject during pregnancy and only three of the 21 were smokers.

No subject smoked a cigarette in the 12 h before the tests. The serial measurements were made in each subject at an identical time of day, although certain subjects were tested in the morning (09.00–12.00 hours) and others in the afternoon (14.00–17.00 hours).

Single-breath transfer factor measurements were carried out by the method of Ogilvie, Forster, Blakemore & Morton (1957). The test gas contained (by vol.) carbon monoxide (0.3%), helium (15%), oxygen (20%) and a balance of nitrogen. Carbon monoxide concentration was measured by an infrared analyser (Infra-Red Developments Ltd), and relative helium concentrations by a catharometer (Cambridge Instruments). Residual volume was calculated from the reduction in helium concentration in a single inspiration of the test gas. The lung volume at which measurement of the transfer factor was made was calculated by adding this residual volume to the inspired volume. The breath-holding time was acceptable if between 9 and 12 s. Residual volume was also measured on each subject by the helium dilution method (Meneely & Kaltreider, 1949). The transfer factor measurement was made on at least three occasions on each subject at each visit and the average of three satisfactory measurements calculated.

At each visit, 17β-oestradiol was assayed in venous plasma with the modification by Dodson, Couffts & Macnaughton (1975) of the method of Wu & Lundy (1971). Haemoglobin concentration was also measured by an automated electronic counter (Coulter S). Each subject was questioned on respiratory symptomatology at each visit, a modification of the MRC questionnaire being used. Comparisons were made by paired t-test.

Results

Detailed results are given in a Table deposited as Clinical Science and Molecular Medicine Table 77/9 with the Librarian, the Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE, who will issue copies on request.

Serial measurement of transfer factor

Transfer factor measurements were significantly higher at the initial study than the measurements in the non-pregnant state \((P<0.001)\), but fell significantly during pregnancy (Fig. 1). The measurements at the second study were significantly less than those at the first \((P<0.001)\) and a further fall occurred between the second and third visits \((P<0.005)\). The lowest mean value was at 24–27 weeks gestation with no further significant fall thereafter. The transfer factor as measured post

![Fig. 1. Mean values for transfer factor \((T1)\) throughout pregnancy and post partum. ▲, \(T1\) measured; ○, \(T1\) corrected for haemoglobin and alveolar volume.](image-url)
partum was slightly but significantly greater than that measured between 36 weeks and term ($P < 0.001$).

**Measurements of haemoglobin concentration**

A significant fall in haemoglobin concentration occurred between the first and third studies ($P < 0.01$) and thereafter no further significant change took place. The lowest mean value occurred at 20–23 weeks gestation. The post-partum values were significantly higher than those recorded at the end of pregnancy ($P < 0.001$).

**Measurements of alveolar volume**

There was a small, significant change in the alveolar volume as measured by the dilution of helium in the test breath during pregnancy. The mean values at 24–27 weeks ($P < 0.001$), 28–31 weeks ($P < 0.05$) and 32–35 weeks ($P < 0.001$) were significantly less than those in the first trimester. The mean differences were, however, small and the maximum mean difference of 0.46 litre was between 8–11 weeks and 32–35 weeks.

**Corrections of transfer factor measurements for changes in haemoglobin concentration and alveolar volume**

The measured transfer factor values were corrected for changes in haemoglobin concentration by the correction formula of Cotes, Dabbs, Elwood, Hall, McDonald & Saunders (1972) (Fig. 1). In correction of the transfer factor for changes in alveolar volume it has been assumed that a change in lung volume of 1 litre gives a change in transfer factor of 0.867 mmol min$^{-1}$ kPa$^{-1}$ (Cotes & Hall, 1970). The mean alveolar volume for each subject during all tests was calculated and the measured transfer factors were normalized to this volume. Even with such corrections the values in the first trimester were still significantly higher than those when non-pregnant ($P < 0.005$). A significant fall in transfer factor was still observed between 8–11 weeks and 24–27 weeks ($P < 0.005$), but the post-partum values were not significantly higher than at the end of pregnancy.

**Measurements of transfer coefficient ($KCO_{2}$)**

The transfer coefficient was calculated from the transfer factor measurements that had been corrected for alteration of haemoglobin concentration. There was a significant fall ($P < 0.001$) in the mean transfer coefficient in the first trimester compared with that in the non-pregnant state, but thereafter this fall during pregnancy was not so marked and only between 36 weeks and term were the values significantly lower than those in the first trimester ($P < 0.05$). There was no significant difference between the measurements at the end of pregnancy and those post partum. The complete data for measurements of transfer factor, alveolar volume, haemoglobin concentration, transfer coefficient and corrected transfer factor, for each subject at each visit, are given in the deposited Table referred to above.

Measurements of plasma 17β-oestradiol serially throughout pregnancy in the 21 subjects (Fig. 2) show no obvious direct relationship between changes in the concentration of plasma 17β-oestradiol and changes in transfer factor. The time-course of the changes in the concentration of plasma 17β-oestradiol during pregnancy is similar to that in other published data (Tulchinsky, Hobel, Yeager & Marshall, 1972; Smith, 1966).

**Discussion**

Hormone-mediated changes can be demonstrated to occur in many physiological systems during
pregnancy (Hyttén & Leitch, 1971). The alteration in transfer factor \((T1)\) during pregnancy, which we have found, may be secondary to alterations in the known factors determining the transfer of carbon monoxide across the alveolar capillary membrane rather than a direct effect of foeto-placental hormones on this process.

The transfer factor for carbon monoxide is dependent on the membrane diffusing capacity \((D_{M})\), the pulmonary capillary blood volume \((V_{C})\) and the reaction rate of carbon monoxide with haemoglobin \((\theta)\). This relationship is expressed by eqn. (1) (Roughton & Forster, 1957).

\[
\frac{1}{T1} = \frac{1}{D_{M}} + \frac{1}{\theta V_{C}}
\]  

(1)

The numerical value of the reaction rate \(\theta\) is dependent on the haemoglobin concentration \((Hb)\), the mean pulmonary capillary \(P_{O_2}\) \((Pc,O_2)\) and the ratio of the permeability of the erythrocyte membrane to the permeability of the erythrocyte's interior \(\lambda\) (Roughton & Forster, 1957). During pregnancy each of these dependent variables may be altered. The haemoglobin concentration falls during the course of a normal pregnancy (Hyttén & Leitch, 1971), as we confirm. Mean capillary \(P_{O_2}\) may also vary throughout pregnancy, due to the fall in alveolar \(P_{CO_2}\) that is known to occur (Lyons & Antonio, 1959; Pernoll, Metcalfe, Kovack, Wachtel & Dunham, 1975). This fall was only about \(0.5\) kPa in the latter study. In addition, direct measurement of end-tidal \(P_{O_2}\) serially in a number of subjects during pregnancy (J. A. Milne & A. I. Pack, unpublished work) indicates that there is no significant change in this variable after the first trimester. The ratio of permeabilities \(\lambda\) is altered in pregnant ewes (Nicolson & Roughton, 1951), but the average value of \(\theta\) in the pregnant ewes is identical with that found in non-pregnant humans (Roughton & Forster, 1957). Consideration of the original data on numerical values of \(\theta\) indicates that during pregnancy alteration of haemoglobin concentration will cause the largest variation in \(\theta\).

The effect of alteration of the reaction rate on measurement of transfer factor can be assessed by reformulation of eqn. (1), i.e.:

\[
T1_{N} = \frac{\theta + D_{M}V_{C}}{\theta_{N} + D_{M}V_{C}} \cdot T1
\]

(2)

where \(T1_{N}\) is the transfer factor which would result from alteration of the reaction rate from \(\theta\) to \(\theta_{N}\), the transfer factor when the reaction rate is \(\theta\) being \(T1\). Since the largest single factor altering is variation in haemoglobin concentration in our study, we follow Cotes et al. (1972) in assuming that \(\theta\) is linearly related to haemoglobin concentration and is unity when \(Hb = 14.6 \text{ g/dl}\). The effect of other factors is comparatively much smaller. With these assumptions we obtain the relationship in eqn. (3), which we have used to correct the measured values of transfer factor for alterations in haemoglobin concentration. It is assumed that \(D_{M}/V_{C} = 0.7\) (Cotes et al., 1972).

Our use of eqn. (2) and eqn. (3) means that when \(\theta < 1\) at a haemoglobin of \(14.6\) g/dl, the actual transfer factor would have been greater than we obtained.

An alternative correction equation (eqn. 4) for normalizing for alteration in haemoglobin concentration is that proposed by Dinakara, Blumenthal, Johnston, Kauffman & Solnick (1969).

\[
T1 \text{ (correction)} = T1 \text{ obs.} / 0.06965 [Hb]
\]

(4)

Eqn. (4) was derived empirically from transfer factor measurements in 50 subjects with various haemoglobin concentrations, on the assumption that the transfer factor in all subjects would be 100% of a predicted normal value if the haemoglobin concentration were normal. The predicted normal values were obtained in a study of a control group of subjects for whom no details are given on haemoglobin concentration. Eqn. (4) normalizes the transfer factor to that which would be present at a haemoglobin concentration of \(14.4\) g/dl (0.06965 \times 14.4 = 1.0). Although the magnitude of the correction introduced by eqn. (4) is greater than that made by eqn. (3), application of eqn. (4) to our results does not alter the observed trend in transfer factor changes during pregnancy.

Both eqn. (3) and eqn. (4) are strictly inapplicable to correction of the observed results for variation in haemoglobin concentration, as one represents a slight but unknown under-estimation of the effect, and the other has an
inadequate scientific basis. Nevertheless the size of the changes in transfer factor during pregnancy, even after correction for variation in haemoglobin concentration, are such that they are not likely to be explained by the inadequacy of the correction formulae.

The transfer factor measured by the single-breath method is also affected by alterations in the lung volume at which the measurement is made (Cotes & Hall, 1970). Applying these published data to the present work shows that the fall in transfer factor cannot be explained solely by the measured fall in lung volume. The relative lack of change in the transfer coefficient in the presence of a significant fall in transfer factor (independent of lung volume) is explained by the fact that the calculation for the transfer coefficient \((T_L/V_A)\) over-compensates for the fall in lung volume.

It is also necessary to consider the possibility that the observed changes in transfer factor might be related to the known changes in cardiovascular function during pregnancy. The cardiac output rises during pregnancy (Hamilton, 1949; Lees, Taylor, Scott & Kerr, 1967) by about 1.5-2.0 l/min. From a theoretical viewpoint, if carbon monoxide uptake \((V_{CO})\) is considered as being limited both by perfusion \((\dot{Q})\) and diffusion \((D)\) then

\[
\dot{V}_{CO} = \dot{Q}BP_{a} [1 - \exp (- D/\dot{Q}\beta)]
\]

(from Piiper, Canfield & Rahn, 1962), where \(\beta\) is the slope of the carbon monoxide dissociation curve of blood and \(P_{a}\) is the alveolar partial pressure of carbon monoxide. This equation indicates that for a 2.0 litres change in cardiac output, the transfer factor would change by only 0.02 mmol min\(^{-1}\) kPa\(^{-1}\). The lack of effect of changes of cardiac output on transfer factor has been demonstrated experimentally (Ross, Frayer & Hickam, 1959; Turino, Brandfonbrener & Fishman, 1959). No change in transfer factor is observed in subjects with hyperthyroidism after treatment (Johnston, Stein & Kimbel, 1958). Thus the rise in cardiac output in pregnancy will not alter the transfer factor for carbon monoxide.

The pulmonary capillary blood volume \((V_C)\) may be increased in pregnancy since both cardiac output and plasma volume rise (Hyttten & Leitch, 1971), and also \(V_C\) is known to be increased both in the second half of the men-


