The Physiology of Human Pregnancy

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BLACKWELL
SCIENTIFIC PUBLICATIONS
OXFORD

FIRST PUBLISHED 1964

Printed in Great Britain by

WILLMER BROTHERS AND HARAM LTD, BIRKENHEAD

and bound by

THE KEMP HALL BINDERY, OXFORD

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Foreword

Until about thirty years ago, the most urgent problem facing the obstetrician was the high rate of maternal mortality, mainly from infection, haemorrhage and obstructed labour. The antibiotics have brought infection under control; haemorrhage is much less dangerous, thanks to blood transfusion; and difficult labour can be safely avoided or overcome by caesarean section. The reduction of maternal mortality to a small fraction of what it used to be has been accompanied by a less dramatic yet substantial fall of perinatal mortality, brought about mostly by a reduction of deaths from birth trauma. The main causes of foetal wastage are now abortion, premature birth, defective foetal growth, congenital malformations and such serious complications as antepartum haemorrhage and hypertension. It seems possible that many of these pathological states encountered in clinical obstetrics result from failure of the mother to achieve the adaptive changes needed. We therefore need to know much more about the changes in body composition and function which occur in human pregnancy.

In animal studies the physiologist would probably use a carefully chosen inbred strain of animal with a high reproductive performance and maintain it in uniform and healthy conditions. In the human it is clearly impossible to have such well controlled conditions and this imposes severe limitations on the scope of work in the human field. It is impossible at present to allow for genetic factors nor can environment be controlled accurately. Epidemiological research has shown, however, that some groups of women are more vulnerable in pregnancy than others. For example, the risks of difficult labour and of stillbirth from all causes, are much higher in a first than a second or third pregnancy and in 'elderly' primigravidae. At any age and parity risks are greater in women from the lowest socio-economic groups. The social gradient is associated with gradients in physique and in general health, women from the more

affluent classes being, on the average, taller and healthier than those from the poorer classes.

In studies which deal with the 'normal' or physiological changes in the mother during pregnancy, great care must be taken to select as the subjects for investigation women who are most likely to show the highest level of reproductive efficiency. For example, primigravidae should be between the ages of about twenty and thirty, tall and in good health and, if possible, from the upper socio-economic groups. This, in practical terms, is as near as we can get in the human to the more stringent procedures used in similar studies in animals.

Our epidemiological studies have shown, however, that even in such carefully selected women obvious differences in response to pregnancy occur. For example, there is considerable variation in the amount of weight gained during pregnancy and this is associated with differences in perinatal mortality and prematurity rates. It is therefore of great importance when drawing conclusions from an intensive physiological study of a few patients to have an accurate epidemiological study of the total population from which the patients are drawn. Again it is most important for the physiologist to work in an obstetrical atmosphere, since not only may the normal physiological response to pregnancy give insight into the aetiology of the pathological but the pathological response may help us to understand the normal.

How is this work to be organised? Suitable patients, when chosen, must be persuaded to undergo procedures which call for their active co-operation. Even the sampling of blood from a superficial vein at regular intervals is not quite painless, and usually requires that the patient must attend a clinic under specified conditions. She must be told that measurements are being made for research purposes, and are unlikely to benefit her directly. Nevertheless, she expects some 'reward', if only unconsciously, and it is necessary to stage research in circumstances where the highest standards of clinical care can be offered, not only in terms of obstetric techniques, but also in terms of personal attention. Either the research physiologist must himself be a competent obstetrician, or he must work in close co-operation with his obstetric colleagues.

Beds should be set aside in hospital so that when the needs of research require it, patients who otherwise do not have to be in hospital, can be admitted. In this way the best timing of observat-

ions is made possible by ensuring that a bed is available when it is required. In the Aberdeen Maternity Hospital eight beds have been set aside for research purposes alone. Care is taken to give patients in these beds an exceptionally high standard of comfort and personal attention, for the severely practical reason that research plans often make it necessary to persuade women to stay in hospital for longer periods than is clinically essential. The special 'research beds' are serviced by a group of nurses who are trained in unusual procedures, and who recognise the need for meticulous accuracy. In addition to beds, a research unit devoted to the physiology of pregnancy needs out-patient facilities and it is convenient to have the laboratories adjacent to the clinical accommodation.

The assembly of the proper team essential to research into both the physiological changes in pregnancy and the significance of deviation from the 'normal' presents the difficulties common to all clinical research in depth in any field. The solution to the problem must depend on circumstances. Few practising obstetricians have the time or the special experience that is necessary for many forms of physiological investigation. On the other hand, it is not easy to persuade professional physiologists to leave their classrooms and laboratories to face the difficulties of working with human subjects. If they are attached to the obstetric staff when at a relatively junior stage, they may come to feel that they are isolated from their own profession. Yet the study of human physiology requires prolonged devotion to it. In Aberdeen, we have been fortunate in obtaining the support of the Medical Research Council which by establishing a research unit in a maternity hospital is able to offer career posts to staff who are not obstetricians. The possibility of isolation is reduced by a system of joint appointments between the research unit and the appropriate University departments.

It is becoming more and more difficult to study 'normal' pregnancy since the intensification of medical care in an attempt to reduce mortality and morbidity to a minimum may lead to forms of treatment which modify 'natural' response to pregnancy. A few examples are prophylactic use of iron or folic acid, the giving of other vitamin preparations, calcium or diuretics, or the restriction of weight increase by dietary control.

The needs of therapy and research call for close co-operation between physiologists and obstetricians so that on the one hand the patient, on whom difficult and elaborate measurements have been

made, is not given advice or treatment which may change the whole situation; on the other there must be no delay in instituting treatment which the clinician thinks is necessary. Changes in treatment will give rise to fresh physiological problems. For example, if a patient is made to lose weight by eating less, is she losing fat or water or both? Does dietary restriction affect the growth of the foetus and if not why not? If it is beneficial in clinical terms, why is this so? In this connection a great deal of work needs to be done in the evaluation of conventional methods of treatment during the antenatal period so that the research outlook should permeate the whole hospital. Records of all cases must be carefully planned and systematically compiled with a view to subsequent analysis.

Perhaps the most interesting material in this book is its account of the adaptations that take place during the course of pregnancy. Many of them occur so early that they cannot be responses to an immediate need, but must represent fundamental changes that are necessary to secure the safe development of the foetus at a later stage of gestation. To unravel their nature and to determine their meaning will be a prolonged but fascinating task. Meanwhile the authors have made a notable contribution to these fundamental problems not only by their valuable critical review of the literature but also by much original work.

Further progress in obstetrics must be founded on an understanding of the true nature of the physiological processes involved in pregnancy so that discoveries in other fields of medicine, chemistry or pharmacology may be applied rationally.

I therefore hope that this book will be read and carefully studied by practising obstetricians and all others interested in human reproductive physiology.

DUGALD BAIRD

Preface

In his foreword, Professor Sir Dugald Baird has outlined the background against which this book has been thought out and written. It remains for us only to say a little about the book itself.

This is not a review textbook. Certainly it is a synthesis of research and clinical observation, but the literature cited and the research results used to explain a finding or support a point of view are not usually all that might be quoted. We believe we cannot be accused of unfair selection, but the selection has been *ad hoc*. We have been critical and we hope provocative. A distressingly high proportion of published studies are worthless simply because the techniques used have been either intrinsically poor, or unsuitable for pregnancy. For this reason we have placed great emphasis on technical method. We do not wish to encourage an interest in method for its own sake, but in pregnancy perhaps more than in some other situations it is important to consider results in the light of how they were derived.

We have deliberately restricted our field to the physiology of the mother in pregnancy, excluding labour which deserves a book to itself, and the still poorly studied winding-down processes which follow delivery. And except where reference to the foetus was needed to clarify the maternal situation we have made no attempt to include foetal physiology.

It will be plain to those who are kind enough and patient enough to read the book that there are two main themes: first, measurements, and second, requirements and their satisfaction. The first twelve chapters are devoted primarily to measurements of the pregnant woman and of the changes that take place in her physiological processes and metabolism; the last two to the specific requirements that may be derived from these changes and the environmental circumstances in which those needs are to be met.

We have written the book primarily for the interested obstetrician, particularly registrars engaged on research problems; but

we hope too that it may appeal to others, from the more interested medical student to the professional physiologist.

The growth of ideas is always slow and uncertain compared to the prodigiously rapid growth of facts. Ideas and concepts we have developed here have evolved not only from years of personal research and the assimilation of published research results but also from talking and arguing with many people. To all of them we owe some debt of gratitude. Here we can only give our thanks to those who have helped us directly by criticizing the manuscript and giving advice during the writing of the book or by giving us their data. Dr Angus Thomson has been of inestimable help in detailed criticism of the whole book; others have helped with specific sections: Dr S.G.Anderson, Dr S.Aboul-Khair, Dr J.B.Brown, Dr P.S.Brown, Mr W.Z.Billewicz, Dr J.M.Crawford, Dr H.F.Helweg-Larsen, Dr A.I.Klopper, Dr B.F.Matthews, Dr D.R.Mishell, Dr D.B.Paintin, Dr R.V.Short, Dr J.M.Stowers and Dr W.A.W. Walters.

Above all, our gratitude is due to Professor Sir Dugald Baird for creating the environment that made the book possible.

Finally we are grateful to Miss Margaret Porter who has devoted so much of her spare time to typing and retyping the manuscript.

Aberdeen Maternity Hospital F.E.H.
October 1963 I.L.

Contents

CHAPTER 1

The Volume and Composition of the Blood

BLOOD VOLUME

In a paper describing one of the earliest attempts to measure the increase of blood volume in human pregnancy, Miller, Keith and Rowntree (1915) stated that the 'plethora' of pregnancy had been recognised early in the nineteenth century and quoted German work as far back as 1854 which showed a rise of blood volume in pregnant laboratory animals. But until the early 20th century the evidence for plethora in women rested primarily on the demonstration of a reduced concentration of solids and cells in the blood.

The measurement of blood volume

Both the major components of blood, the red cells and the plasma, may be measured by methods based on the dilution principle, but they have seldom been measured together. Usually one is measured and the other is estimated from packed cell volume. The volume of white cells is usually ignored.

PLASMA AND SERUM

A great deal has been written about techniques of measuring the volume of plasma or serum and Gregersen and Rawson (1959) have recently made a detailed review. We will attempt no more than a description of the main principles. In theory any substance that spreads evenly in the plasma and does not leak out of the blood vessels during the time required for mixing is suitable for use and many such substances have been tried. Only two or three have come into common use. Evans blue dye (T1824) has been, and probably still is, the most popular. It fulfils the theoretical requirements of a tracer by attaching itself firmly and selectively to plasma albumin, and the amount that leaves the circulation during the mixing phase is negligible. About 70 per cent has disappeared in 48 hr (Wyers and van Munster, 1961) so that plasma

volume may be measured again after such an interval. Equilibration in the plasma is complete in less than 10 minutes after injection of the dye (Strumia, Colwell and Dugan, 1958) and the final concentration can be estimated on a single sample, taken at 10 minutes, or by extrapolating to zero time the results for a number of samples taken between, say 10 and 30 minutes. In theory, the extrapolation method, which takes account of dye lost from the circulation during the mixing phase, is the more accurate and is preferred by many of the most experienced workers in the field (for example, Reeve, 1947–48; Gregersen and Rawson, 1959); but a careful comparative study by Senn and Karlson (1958) suggests that the method with one ten-minute sample, which is much more convenient in clinical practice, has an error which is no greater than that of the extrapolation method. Caton *et al.* (1951) suggested that 10 minutes was insufficient for complete mixing of the dye in pregnancy and they allowed 15 minutes. Other studies, for example that of Roscoe and Donaldson (1946) and an unpublished Aberdeen study, have shown complete mixing in 10 minutes and that time is now almost universally accepted as sufficient.

Most workers use anticoagulant in the blood samples and are therefore estimating plasma volume. Direct reading of the concentration of dye in plasma or serum is subject to errors due to the presence of fat, to haemolysis and to changes in plasma colour. For this reason many workers have abandoned direct reading in favour of one of the many methods (for example that of Allen, 1951) by which the blue dye is extracted from the plasma on cellulose columns and eluted in an aqueous solution. Recovery of the dye is not complete nor of constant proportion, but tends to be about 97 per cent.

The use of serum instead of plasma reduces to a minimum the errors due to lipaemia (Murray and Shillingford, 1958) and, in our experience, if the subject is fasting and if blood samples are taken with care, direct reading of the dye in serum, taken 10 minutes after injection and compared with standards made up in blank serum from the same subject, gives results of a consistency which compares favourably with that of any of the more sophisticated techniques. Repeated estimates of the serum volume in any one subject should not show a range of more than 5 per cent from the mean.

Another tracer which is widely used to estimate plasma volume is albumin labelled with ^{131}I, that is radioactive, iodinated serum albumin (RISA). This method has its own difficulties (see Gregersen and Rawson's review) but has the advantage that the state of the plasma does not affect the count of radioactivity as it may affect the reading of a dye concentration. A number of workers have compared RISA with Evans blue dye, for example Senn and Karlson (1958) and Overall and Williams (1959). The two methods they say give much the same answer, but Senn and Karlson claim that there is a greater error in the Evans blue method because of variations in the optical density of plasma. Nor is that the only trouble.

From an analysis of results published by Zipf, Webber and Grove (1955) and Inkley, Brooks and Krieger (1955) Overall and Williams concluded that 'techniques of estimating plasma volume are significantly influenced by the technicians employing them. Evans blue dye and RISA are not two standard techniques but instead represent two families of techniques, members of which must be identified according to the investigators using them'. This is an important conclusion which might explain in part the wide range of mean values for plasma volume found at different stages of normal pregnancy by different workers. In any case it is clearly unwise to compare directly estimates made in one laboratory with estimates made in another by a technique broadly similar but differing in many details. Fortunately for our purposes in this book, the absolute values for plasma volume are of less interest than the extent of the change during pregnancy.

There are other methods of measuring plasma volume but less is known of their characteristics. Many different tracers have been used; for example Geigy Blau 536 is popular in continental European countries, and recently dextran and iron-dextran as described by Semple, Thomsen and Ball (1958) and Mackenzie and Tindle (1959).

None of the tracers used in the measurement of plasma volume crosses the placenta, at least during the time needed for equilibration in the maternal blood. Measurements in pregnancy are therefore of maternal plasma only.

RED CELL VOLUME

In the earliest method of measuring red cell volume haemoglobin

was labelled with carbon monoxide (CO). It has seldom been used in pregnancy and has two characteristics which make it difficult to compare its results with those of other methods. First, carbon monoxide has been shown by Nomof *et al.* (1954) to overestimate red cell volume by 12 to 16 per cent when compared with direct labelling of cells (see below), because it attaches itself to myoglobin and other body pigments; second, it crosses the placenta and the measurement therefore includes foetal haemoglobin and myoglobin. Since radioactive labels suitable for red cells became available in the 1940s, the use of CO as a label has almost disappeared. The current application of the dilution principle to the measurement of red cell volume requires labelled red cells which can be injected into a subject in known quantity and may be easily identified in a sample of blood after mixing has taken place.

Radioactive isotopes of iron, ^{55}Fe and ^{59}Fe, have the advantage of being built into the haemoglobin molecule; they cannot be eluted and lost from the circulation during equilibration. But they have two great disadvantages: (1) donor cells are needed and there is therefore a risk, however careful the cross-typing may be, of the clumping or lysis of the injected cells and (2) there are the hazards of a long-lived source of radiation (^{55}Fe has a half-life of nearly 3 years, ^{59}Fe of 45 days) which cannot be excreted. The latter risk is greater when these isotopes are used in pregnancy because some is transferred to the foetus. They are now seldom used to label cells.

The two most popular techniques at present are the *in vitro* labelling of a sample of the subject's own red cells with ^{51}Cr or ^{32}P. Considerable experience with these isotopes has been amassed and their characteristics are now well understood. Chromium is the better tracer and is lost from the cells at the rate of only 1 per cent per day compared to a rate of about 6 per cent per hour for phosphorus. The chromium labelling technique has been described in detail by Nomof *et al.* (1954).

TOTAL BLOOD VOLUME

Without doubt the best estimate of total blood volume is obtained when plasma volume and red cell volume are measured simultaneously, say with Evans blue dye and red cells labelled with ^{51}Cr. But the two measurements are rarely made together and the majority of published reports of blood volume in pregnancy are based on estimates of either plasma volume or red cell volume. The

fraction not directly estimated is calculated from an estimate of the proportion of red cells in the blood, which proportion is often termed the 'body haematocrit'. That term is now in the literature although the expression 'over-all cell percentage' used by Gregersen and Rawson (1959) is more specific and to be preferred. The over-all cell percentage in blood is derived from the cell percentage (packed cell volume or haematocrit) of a sample of peripheral venous blood. Two corrections must be made. The first is purely technical: plasma is trapped between the red cells in the packed cell column of the centrifuged blood and may cause an over-estimate of the cell percentage by up to 5 per cent. The value depends on the centrifugal force and time of centrifugation; with some modern high speed equipment there may be no appreciable trapping of plasma.

The second correction is necessary since the cell percentage in venous blood and all large vessels is higher than the over-all cell percentage because of the relatively high proportion of plasma in the smaller blood vessels and capillaries. The physics of blood flow is such that plasma tends to line the vessel wall while red cells congregate and flow in the centre of the vessel. The sorting out is negligible in large vessels but becomes important in small vessels and capillaries where there is a relatively wide zone of plasma surrounding a concentrated core of more rapidly moving red cells, which results in a permanently increased proportion of plasma in small vessels. The effect is accentuated when red cells tend to clump as they do in pregnancy (see below). Chaplin, Mollison and Vetter (1953) found that the over-all cell percentage in non-pregnant adults was 91 per cent of the venous cell percentage (i.e. the 'haematocrit ratio' was 0.91) when the red cell volume was estimated with cells labelled with ^{32}P and the plasma volume with Evans blue dye by a technique that involved correction for the dye leaving the circulation during equilibration. Where plasma volume and total red cell volume have been measured simultaneously in pregnancy by Caton *et al.* (1951) and by Verel, Bury and Hope (1956) the ratio between the over-all and the venous cell percentages varied widely; the range found by Verel *et al.* for 13 subjects was from 87 per cent to 103 per cent, and Caton *et al.* found a range of from 71 per cent to 124 per cent. More recently, Paintin (1963) has shown that, if the total red cell volume is estimated with cells labelled with ^{51}Cr and if the serum volume is