J. Physiol. (1941) 100, 263–274 612.63

THE BEHAVIOUR OF THE PREGNANT UTERUS OF THE GUINEA-PIG

By G. H. BELL

From the Institute of Physiology, University of Glasgow

(*Received* 22 *May* 1941)

THE factors involved in determining the onset of parturition are certainly complex, but an initial simplification can be made if we assume—and there are good grounds for doing so—that they are chiefly hormonal and that the nervous system plays a relatively minor part. In attacking this problem of uterine activity the first step must obviously be the investigation of the spontaneous activity of the uterus at various stages of pregnancy; and in view of the current theories of the role of the posterior lobe of the pituitary gland the reactivity of the uterus to oxytocin should be examined at the same time. The second step should be the investigation of the oxytocic power of the blood throughout pregnancy; but the difficulties here are so great [see Bell & Robson, 1935] that only the first step is as yet possible.

The purpose of this paper is to describe the spontaneous activity and reactivity of the guinea-pig uterus during and after pregnancy. These have been examined in considerable detail in vitro in the human subject [Robson, 1933*b*], in the rabbit [Knaus, 1927, 1928 and Robson, 1933*a*], in the mouse [Robson, 1934], and in the rat [Brooksby, 1937]. Reports of the activity and reactivity of pregnant uteri in vivo are much scarcer, much less complete, and are difficult to locate because the abstracts of reviewers often fail to indicate whether the experiments were carried out in vivo or in vitro. As some early experiments comparing behaviour in vitro and in vivo in the rabbit [Robson, 1935] showed no marked divergence, perhaps this neglect is not to be wondered at. The human uterus has been studied in vivo by Bourne & Burn [1927] and Moir [1934], the rabbit uterus by Knaus [1926] and by Reynolds & Firor [1933] (motility only), and the cat uterus by Robson & Schild [1938]. The information concerning the behaviour of the pregnant human uterus in vivo is still fragmentary.

Methods

It was not found practical to obtain pregnant guinea-pigs by merely putting a male with the female at oestrus. This is not surprising since it is now known [Blandau & Young, 1939] that the period during which the ovum can be fertilized is very short. Each male was allowed to run with three or four females in a single cage; the females were examined once daily and the times at which the vaginae were open were recorded. The vaginae were open for 3 days on the average, with a range of 1–7. The beginning of pregnancy was reckoned as occurring at the middle day of the last oestrous period; the error involved in this assumption is small. If a high proportion of pregnancies is required it seems to be essential to handle the animals as little as possible. Occasionally the vagina opened during a pregnancy, but this did not lead to any confusion, as after some experience it was possible to detect a pregnancy by palpation from 3 weeks onwards and to estimate roughly its duration. Ishii [1920] has described a swelling of the external genitalia with some secretion in ten out of twenty pregnant guinea-pigs which occurred usually about 15 or 30 days after mating. It may be that the opening of the vagina during pregnancy is associated with a wave of follicular growth as described by Loeb [1911]. It seems that the fundamental sex rhythm is not completely suppressed during pregnancy in the guinea-pig; this might also be said of man and *Macacus*, where menstruation (though not strictly analogous with oestrus) is occasionally observed in early pregnancy.

At the time of the experiment each animal was anaesthetized with ether followed by chloralose (7 mg./kg. subcut.) repeated as required. A large number of anaesthetic deaths occurred in early pregnancy, but animals more than 1 month pregnant rarely gave any trouble. The external jugular vein was cannulated; the abdomen was opened and a boat-shaped cannula [Bell & Robson, 1936] was attached to the pregnant horn of the uterus without disturbing the foetus. The movements were recorded on smoked paper by a lever connected by a thread running over two pulleys to the centre of the portion of the uterus under the cannula. In the early pregnancies the cannula was chosen so that it spanned one foetus without compression or alteration of the natural position of the uterus; towards the end of pregnancy a cannula 6 cm. long was applied to the uterus over one foetus—thus a record of only a part of the muscle enclosing the gestation sac was obtained. The lack of standardization is more apparent than real because both in early and in late pregnancy a record of only a sample of the uterine muscle is obtained; if the results of

the investigation of numerous samples are consistent the possibility of error is immensely reduced. A similar method—the Cushny myograph—has already been used by Robson & Schild [1938] to investigate the pregnant uterus of the cat; indeed, some such method is the only feasible one if the pregnancy is to be undisturbed during the experiment. That the vitality of the foetus is little disturbed is proved by one experiment of the present series in which the foetuses showed respiratory movements when they were removed from the uterus at its termination. It is difficult, if not impossible, to estimate the resting length of smooth muscle exposed at laparotomy, but every effort was made to keep the tension of the uterine muscle as constant as possible. This was done, when the abdomen was closed, by observing through the glass cannula the amount of raising of the centre point of the uterine muscle by the thread attached to the writing lever. The body temperature was measured by a thermocouple placed in the abdomen, and no observations were made till that was 38° C. The animals remained in apparently good condition for several hours; this was almost certainly due to the complete closure of the abdomen allowed by this procedure. When a satisfactory sample of spontaneous activity had been recorded intravenous injections of specially purified pitocin (kindly supplied by Dr White of Parke, Davis and Co.) were given about 0·5 c.c. of Locke solution followed by about 0·5 c.c. of Locke solution to wash out the cannula. A graded series of injections was given, and the threshold dose was taken as the minimum amount which would produce a small but sustained contraction; usually this was accompanied by an increase in the frequency of the waves so that the pattern of the spontaneous activity was altered. This part of the experiment was not unduly prolonged so that strips of the uterus were obtained in good condition for the experiments in vitro. Two strips were taken from the portion of the uterus to which the cannula had been attached and were suspended in the usual thermostatically controlled bath containing 60 c.c. of oxygenated Locke solution.

Results

Typical records from the living animal are shown in Figs. 1 and 2, from early and late pregnancy respectively. The uterus was active, as in other animals, at all stages of pregnancy, and in general the amplitude of the movements increased with the duration of pregnancy, i.e. as the amount of muscular tissue increased. The pattern of the contraction waves in any one experiment is not by any means regular and it is not easy to distinguish tracings (except by amplitude) made early in preg-

nancy from those made towards the end; the mean duration of the contraction waves up to the 30th day was 3·6 min., from the 31st to the 49th day it was 4·7 min., and from the 50th to the end of pregnancy it was 10·4. Owing to the scatter of the observations only the difference between the very early wave durations and the prepartum values are significant (2·23 times the standard error).

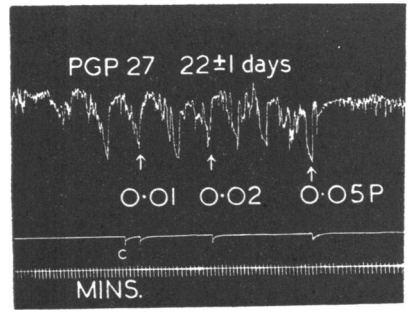

Fig. 1. *PGP* 27, 22±1 days pregnant. Reaction to 0·05 unit of pitocin intravenously; no reaction to smaller doses. At *C* chloralose given subcutaneously.

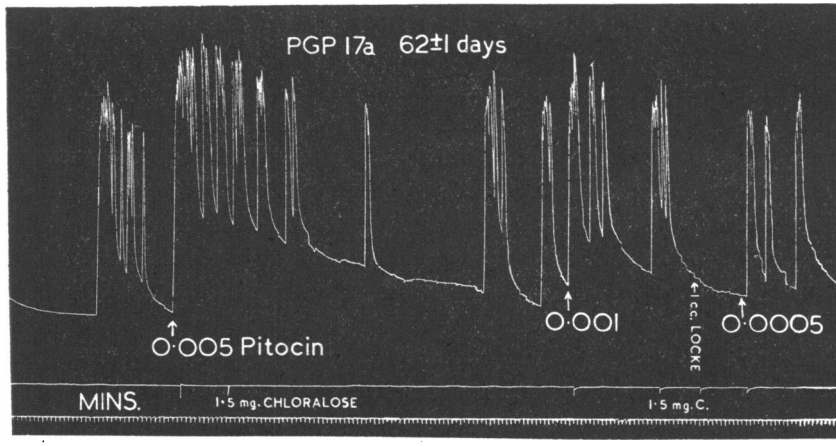

Fig. 2. *PGP* 17*a*, 62±1 days pregnant. Reaction to 0·001 unit of pitocin intravenously; smaller doses of pitocin and control dose of plain Locke solution without effect.

The threshold dose of oxytocin at various stages of pregnancy is recorded in Fig. 3. Because of the wide range of values the dosage has been plotted on a logarithmic scale; this spreads out the lower values and makes their significance clearer. It will be seen that in early pregnancy the uterus is comparatively unreactive, but that it becomes more and

more reactive as the pregnancy proceeds. During the last fortnight the uterus reacts to one-fiftieth of the amount required in early pregnancy.

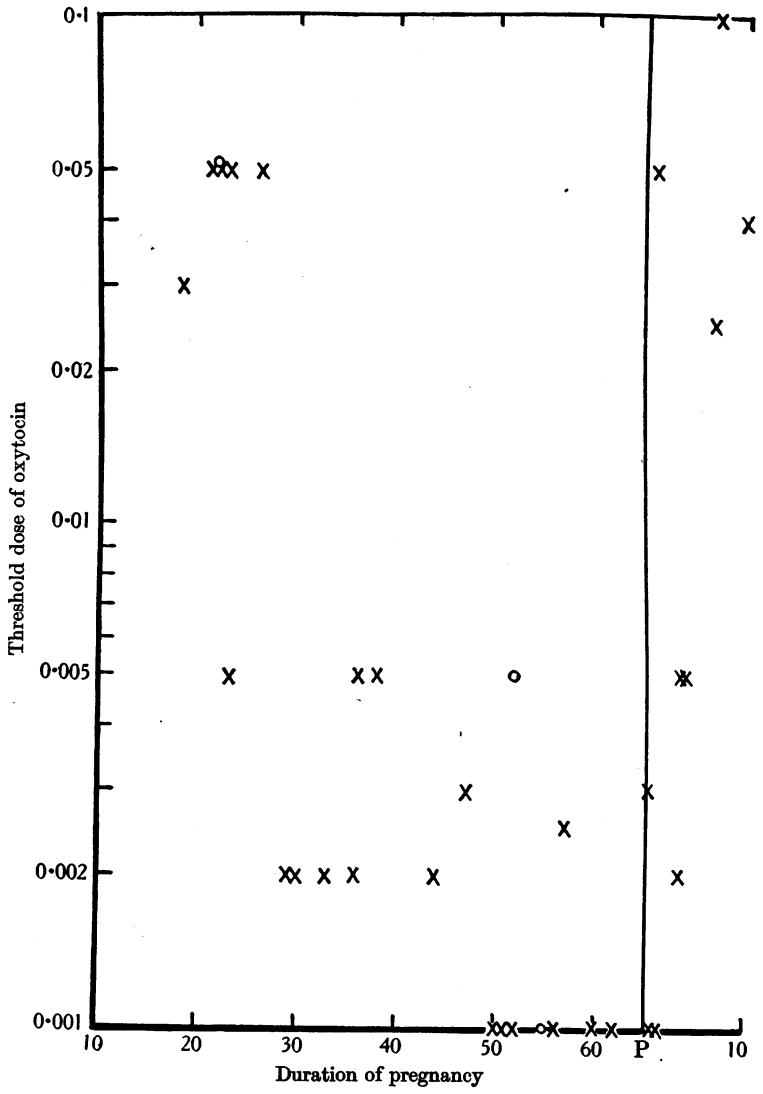

Fig. 3. The threshold dose of oxytocin at various stages of pregnancy. × denotes that the duration of pregnancy was timed from the opening of the vagina; ○ means that the duration of pregnancy was estimated from the graph of Fig. 4.

Very soon after parturition the reactivity becomes once more much less than the prepartum level. There is some variation in the actual weight

of the animals (see Discussion) at the time of experiment even when allowance is made for the weight of their foetuses. The greatest correcting

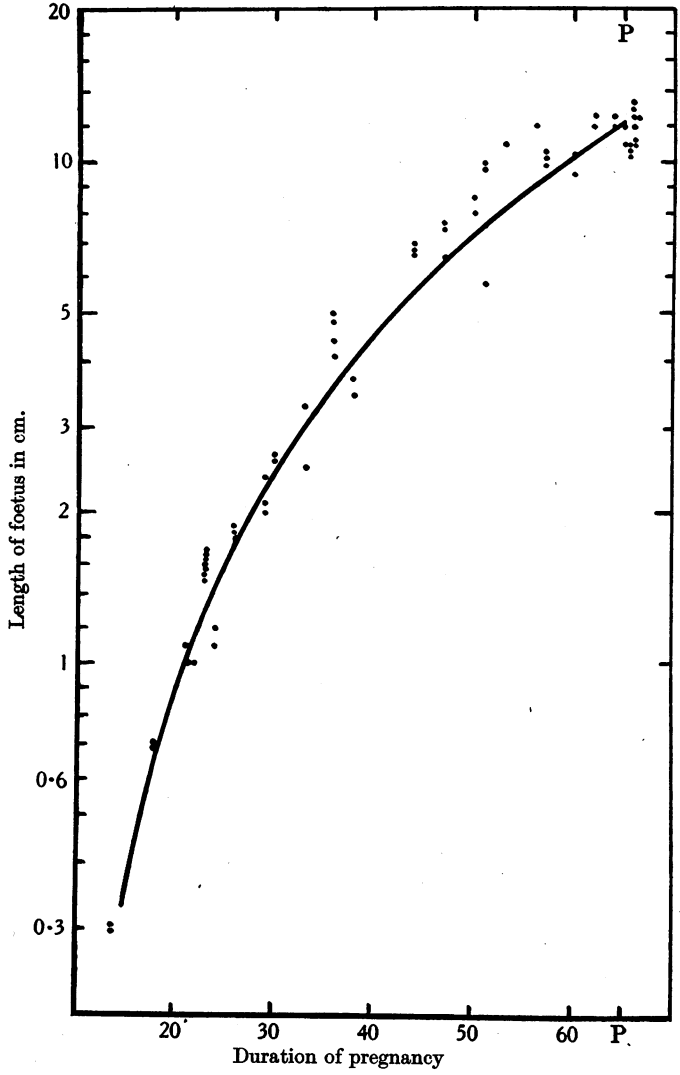

Fig. 4. This graph shows the rump to nose lengths—plotted on a logarithmic abscissa—of all foetuses whose age was known. The line has been drawn to the equation

$$y = -0{\cdot}417 + 0{\cdot}00407x^{1{\cdot}924},$$

where y is the length of the foetus in cm. and x the duration of the pregnancy in days.

factors necessary to express the threshold dose on the basis of the average body weight are 1·5 and 0·7; in most cases it would be, of course, much

less. This is within the range of the experimental error in determining the threshold and therefore the correction has not been made. In any case it would alter very little the general trend of Fig. 3.

When the threshold doses from the in vitro experiments are plotted on a diagram like Fig. 3 there is a very wide scatter of points, and as will be shown in the Discussion very little reliance can be put on them.

The weights of the foetuses in any one litter were very variable, whereas the lengths were remarkably similar. An attempt to find a formula to give the average weight of a litter corrected for the number in it had to be abandoned for lack of information. Fig. 4 gives on a logarithmic ordinate the observed length (nose to rump) of all the foetuses against the duration of pregnancy on the abscissa. The continuous line has been drawn to the equation $y = -0{\cdot}417 + 0{\cdot}00407x^{1{\cdot}924}$, where y is the length of the foetus in cm. and x the duration of the pregnancy in days. This is the simplest form of equation to give a reasonable fit; no physiological significance is to be attached to the constants, the equation obviously cannot hold in early pregnancy and must not be used at durations less than 15 days. This graph (Fig. 4) was used to estimate the duration of pregnancy in some animals; the data obtained in this way are entered as ○ in Fig. 3; an × in Fig. 3 indicates that the duration of pregnancy is reckoned from the vaginal opening. The small degree of scatter in Fig. 4 suggests that timing by vaginal opening is reasonably accurate.

Discussion

Pregnancy in the guinea-pig is maintained as in other species in spite of active movements of the uterus. The results support the theory that parturition is dependent on sensitization of the uterus to oxytocin, but since the uterus shows a high reactivity to oxytocin during the last fortnight of pregnancy this sensitization by itself cannot be enough to bring about parturition. The actual moment of delivery might be determined by a sudden outpouring of oxytocic material. The guinea-pig can maintain a pregnancy and deliver its young after removal of the pituitary gland [Pencharz & Lyons, 1933]; the source of the oxytocic material in a normal delivery is thus not necessarily the pituitary—it may be the hypothalamus, the placenta, or even the foetal pituitary [Bell & Robson, 1937]. This notion of the cause of parturition could be confirmed only if information of the oxytocic property of the blood at various stages were available. But this is an elusive problem which will not be solved until we have a specific test for oxytocin. The difficulty is that the uterus of any animal contracts in the presence of a large number of substances

many of which have obviously no physiological significance [Bell & Morris, 1934; Bell & Robson, 1935].

Before proceeding to discuss the cause of the alteration in reactivity to oxytocin certain fundamental questions must be considered, namely, the manner in which the pitocin reaches the uterus and its concentration when it does so. Since the injection into the jugular vein was always given within a few seconds and in approximately constant volume into an animal with a very short circulation time the oxytocin should be distributed rapidly and evenly through the blood stream; the rate of arrival or impact on the uterus should be about the same in different experiments, always provided that the circulation rate through the muscle remains the same throughout pregnancy. It is well known, however, that the amount of blood in the uterus and the rate of blood flow through it increase as pregnancy advances; but since at the same time the mass of the uterus becomes greater, there may be little alteration in the circulation rate per unit volume of muscle. More important still is the finding [Barcroft & Rothschild, 1932] that the blood volume in the uterus is most closely related to the weight of the placentae; the increased blood flow is needed to meet the requirements of tissues other than the myometrium. Although there is no quantitative information there seems to be no a priori reason to expect that the myometrium during pregnancy—when there is no great activity—should require an increased blood flow; furthermore, it would require a 50-fold increase of vascularity from the 20th to the 50th day of pregnancy to reduce the data of Fig. 3 to a common level. It seems only reasonable then to assume that the alteration in reactivity observed does indicate a real alteration in the state of the uterus. This perhaps academic discussion does not at all affect the main conclusion that during the last fortnight of pregnancy a relatively small quantity of oxytocin is able to produce a marked effect on uterine activity and that parturition could be much more easily initiated then than early in pregnancy.

There remains the explanation of the variation of the sensitivity of the uterus to oxytocin throughout pregnancy. According to Loeb [1906, 1911] the corpus luteum of the guinea-pig is fully formed about 5 days after ovulation and remains till the 40th day when degenerative changes are found in it. It will be seen from Fig. 3 that this marks the middle of the transition period from very low to very high reactivity of the uterus to oxytocin. It has been known for many years that the guinea-pig goes into heat shortly after parturition, and more recently that ovulation with subsequent formation of corpora lutea takes place at this time [see

Parkes, 1929]. The rise in the reactivity of the uterus towards the end of pregnancy is associated in the present experiments with the decline of the corpora lutea of pregnancy, and the post-partum fall in the reactivity to oxytocin is associated with the formation of fresh corpora. The results of Bell & Robson [1936] were taken to show that 'progestin has no appreciable inhibitory action either on the reactivity to oxytocin or on the spontaneous rhythmic activity of the guinea-pig uterus'. At the time of these experiments pure progesterone had just become available in small quantities, and the technique did not allow of so accurate an estimate of the threshold dose as that used in the present series of experiments. The effect of larger doses of progesterone is being investigated at present because if larger doses of this hormone cannot bring about a reduction in reactivity it will be necessary to postulate the action of some other hormone. It may be that the high reactivity at the end of pregnancy is brought about by the decline of luteal activity and at the same time increased oestrin action. The results of Bell & Robson [1936], however, did not show any increase of reactivity in oestrin-treated animals.

The degeneration of the corpus luteum about the 40th day of pregnancy is strikingly confirmed by Pencharz & Lyons [1933], who found that hypophysectomy on the 35th day terminated the pregnancy, while if it were performed on the 41st day it did not cause abortion. Under these circumstances one would expect an immediate degeneration of the corpus luteum, and in fact they found that immediately after delivery the corpus luteum was markedly degenerated. There is very good evidence, however, that the placenta of some animals can produce sufficient progesterone to maintain a pregnancy after ovariectomy [for discussion see Robson, 1940]. This does not seem to have been shown in the guinea-pig—perhaps because evidence of progesterone action cannot readily be obtained in this animal. The present series of experiments fits in well with previous work suggesting the decline of the luteal activity about the 40th day and gives no evidence to show that progesterone is supplied by the placenta.

If this theory of a sudden outpouring of oxytocin is a true explanation of the occurrence of parturition it is difficult to see why a diminution of the reactivity to it in early pregnancy is necessary. It might be argued that, since the outpouring of oxytocin is the actual determining factor the early low reactivity may be an accidental finding. Marrian & Newton [1935] have suggested that, while the uterus is growing, its metabolism may be so altered that it responds more readily to oxytocin. If the data of Fig. 3 are plotted on evenly divided co-ordinates it is found that the

foetus, and therefore presumably the uterus, is growing most quickly when the sensitivity is rising. Curiously enough exactly the opposite occurs when the uterus is involuting; after parturition the reactivity declines very quickly, in some cases it is very low even before the corpus luteum is formed. In the pregnant guinea-pig low reactivity is associated with small fibres—presuming that an increase of uterine size is brought about mainly by an increase in the size of the fibres. Bell & Robson, however, showed that administration of oestrone with or without progesterone produced a small increase in the size of the uterus without alteration of reactivity in the non-pregnant animal; obviously this also requires further investigation. A point in support of this theory of sudden outpouring of oxytocin is that it would explain satisfactorily the finding of Loeb [1923] and Herrick [1928] that removal of corpora lutea in the guinea-pig did not always result in the termination of pregnancy.

In the present work, the marked difference between the concentrations of oxytocin necessary to produce contraction of the uterus in vivo and in vitro, already described for the non-pregnant guinea-pig [Bell & Robson, 1936], has been confirmed. The weights of the pregnant animals varied from 430 to 904 g., with an average of 599 g.; the average blood volume may be taken as 40 c.c. Using this information, it was found that the ratio of the threshold concentration in the blood (i.e. in vivo) to the threshold concentration in the bath of 60 c.c. (i.e. in vitro) varied from 0·1 to 50 with an average of 3·6. Although the discrepancy is less in the present series than in the previous one the conclusion is still that the reactivity of the guinea-pig uterus in vitro is a very unreliable guide to its reactivity in vivo; indeed, it is quite likely that there is no relationship between the in vivo and in vitro findings—in the twenty-five cases in which both experiments were performed the correlation coefficient is $+0{\cdot}24$ with a standard error of 0·20. Furthermore, the type of spontaneous movements given by uterine strips in vitro is quite different to that seen in vivo; the ratio of the duration of spontaneous waves in vivo to the duration of spontaneous waves in vitro varied in the present series from 0·2 up to 10. In spite of doubts which have been raised [Bell, 1941] concerning the reliability of observations made by levers producing tension on the uterus, and in spite of the well-recognized difficulty of the effects of anaesthesia, one is still inclined to believe that records made from the living animal are more likely to represent the actual condition of affairs in the undisturbed animal than are records obtained in vitro. The discrepancies found between the in vitro and in vivo results in the cat forced Robson & Schild [1938] to the same conclusion, which they expressed thus: 'in

certain species it is necessary to investigate the spontaneous activity and the responses of the uterus in the intact animal in order to assess in a satisfactory manner the effects of hormones on the uterus.'

Summary

The activity of the guinea-pig uterus in vivo was examined at various times during and after pregnancy. Spontaneous activity occurred at all times. The threshold dose of oxytocin required to elicit a contraction is high at the beginning of pregnancy and becomes much less in the last fortnight; it rises again shortly after parturition. The threshold dose declines about the time at which the corpus luteum is known to degenerate and increases again when a new corpus luteum is formed. The information at present available does not allow of any explanation of this behaviour in terms of oestrin or progestin. The actual moment of parturition cannot be determined by high uterine reactivity to oxytocin alone, but possibly by a sudden outpouring of oxytocin at a time when the uterus is highly sensitive to it.

A graph with an approximate equation for the estimation of the age of guinea-pig foetuses from their length is given.

The behaviour of the guinea-pig uterus in vitro is an entirely unreliable guide to the behaviour of that organ in the intact animal.

I have to thank Prof. E. P. Cathcart for his interest in this work. The expenses were defrayed by grants from the Rankin Research Fund of the University of Glasgow and from the Medical Research Council.

REFERENCES

Barcroft, J. & Rothschild, P. [1932]. *J. Physiol.* **76**, 447.
Bell, G. H. [1941]. *J. Physiol.* **99**, 352.
Bell, G. H. & Morris, S. [1934]. *J. Physiol.* **81**, 63.
Bell, G. H. & Robson, J. M. [1935]. *J. Physiol.* **84**, 351.
Bell, G. H. & Robson, J. M. [1936]. *J. Physiol.* **88**, 312.
Bell G. H. & Robson, J. M. [1937]. *Quart. J. exp. Physiol.* **27**, 205
Blandau, R. J. & Young, W. C. [1939]. *Amer. J. Anat.* **64**, 303.
Bourne, A. & Burn, J. H. [1927]. *J. Obstet. Gynaec.* **34**, 249.
Brooksby, J. B. [1937]. *J. Physiol.* **90**, 365.
Herrick, E. H. [1928]. *Anat. Rec.* **39**, 193.
Ishii, O. [1920]. *Biol. Bull. Wood's Hole*, **38**, 237.
Knaus, H. H. [1926]. *J. Physiol.* **61**, 383.
Knaus, H. H. [1927]. *Arch. exp. Path. Pharmak.* **124**, 152.
Knaus, H. H. [1928]. *Arch. exp. Path. Pharmak.* **134**, 225.
Loeb, L. [1906]. *J. Amer. med. Ass.* **46**. 416.
Loeb, L. [1911]. *J. Morphol.* **22**, 37.
Loeb, L. [1923]. *Amer. J. Anat.* **32**, 305.

Marrian, G. F. & Newton, W. H. [1935]. *J. Physiol.* **84**, 133.
Moir, C. [1934]. *Trans. Edinb. Obstet. Soc.* **54** 93.
Parkes, A. S. [1929]. *The Internal Secretions of the Ovary.* London: Longmans, Green and Co.
Pencharz, R. I. & Lyons, W. R. [1933]. *Proc. Soc. exp. Biol. N.Y.*, **31**, 1131.
Reynolds, S. R. M. & Firor, W. M. [1933]. *Amer. J. Physiol.* **104**, 331.
Robson, J. M. [1933*a*]. *J. Physiol.* **78**, 309.
Robson, J. M. [1933*b*]. *J. Physiol.* **79**, 83.
Robson, J. M. [1934]. *J. Physiol.* **82**, 105.
Robson, J. M. [1935]. *J. Physiol.* **85**, 145.
Robson, J. M. [1940]. *Recent Advances in Sex and Reproductive Physiology.* London: J. and A. Churchill.
Robson, J. M. & Schild, H. O. [1938]. *J. Physiol.* **92**, 1.