ON PARTURITION AND SOME RELATED PROBLEMS OF REPRODUCTION,

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"But the ultimate aim of all human wisdom is but the liberation of the spirit; and on the degree of this freedom the excellence of all intellectual endeavour must depend. The assembling of facts is a means only; the collector is no more master of his universe than the paving stone is master of the road; he makes it indeed for freer feet to tread. It would be ungrateful to despise his devout and necessary labour; but it is also singularly unfair to limit the majesty of science to so pedestrian a track, and to take from it those ecstasies of the imagination which alone transmute the dead array of facts".

Freya Stark - A Winter in Arabia.

Readers' Union and John Murray, 1941.

We must guard against a fallacy common amongst apologists of science, a fallacy into which, for example, Professor A.V.Hill has fallen, the fallacy of supposing that the men whose work most benefits humanity are thinking much of that while they do it, that physiologists, in short, have particularly noble souls. A physiologist may indeed be glad to remember that his work will benefit mankind, but the motives which provide the force and inspiration for it are indistinguishable from those of a classical scholar or a mathematician.

G.H.Hardy (1940). A Mathematician's Apology.

Cambridge University Press.

TABLE OF CONTENTS

PREFACE

The work described in this thesis has been done during the last seven years. It has been carried out in three universities, in two dairy farms attached to research institutes, and in a maternity hospital. It may perhaps be called an academic piece of investigation but in the doing of it I have at times fought with recalcitrant cows brought into the byre in the middle of the summer rains and at other times I have ausculated foetal hearts in the midst of the startling whiteness of a modern maternity hospital. It is sometimes imagined that the life of a university teacher is cloistered and dull, but it can be made the most interesting, varied, and enjoyable, under the sun.

Sometimes the solution to the particular problem has lain in the proper application of the latest tools of the physicist - but more frequently (for the medical mind still views these with an awe more than tinged with suspicion) the basic methods used were those available to the string and sealing wax physiologists of last century. The young physiologist is apt to think that nothing new can be done without marvellous and complicated apparatus; I have now acquired the greatest respect for those who can tackle fundamental questions in a simple way. One always thinks of Sir Thomas Lewis in this connection - most of

his work on the nociceptive system, for example, was carried out with an ordinary pin.

I find some difficulty in supplying a title to this thesis which will give more than a vague hint as to the ground to be covered - the ground is wide for two reasons. The first is that I have been concerned to make some headway towards a solution to that age old problem - the cause of parturition. It is a problem like the siege of a city with strong defences and boundless resources. Like many others, I have made a direct frontal attack on the problem only to be defeated by the bocby traps before reaching the edge of the objective. There seems to be no course open but to attack the city from all sides, probing for weak spots; but there are up to now no indications of an early capitulation. But this is not the place for a pilgrim's progress to the city of parturition - let us drop the metaphors. The chief difficulties are the want of specific tests for the various hormones and the great differences between different species; this latter difficulty is most disconcerting when one is in search of generalisations and it has necessitated repeating the same piece of work on several types of animals. The process of parturition, the birth of a more or less mature animal out of the uterus, is seen in very many different species - and, superficially at least, it is not so very different from the even more widespread habit of laying eggs.

"That both the Hen and Housewife are so macht,
That her Son born, is only her Son hatcht".

(from verses prefixed by Llewellyn to his translation of "De Generatione Animalium" 1653")

This process must have arisen far back in evolution and since it is outwardly similar in nearly all mammals there is no good reason to suppose that different animals have elaborated different methods. Yet, as will be seen, even such a small group as the rodents shows quite marked divergencies betweer its members, ard in many details they are different from the primates which again do not agree among themselves in the minutiae of their reactions. The variations will, in my opinion, eventually be explained as modifications, brought about perhaps by nutritional differences, of a fundamental pattern which still eludes us. The second reason for the relatively wide scope of this thesis is the wide scope of the subject of reproduction - reproduction is itself a complicated enough process but its influence on the whole body and the influence of the body and even the mind on reproduction must be kept in mind. The investigation of any part necessitates inevitably enquiry into collateral as well as subsequent problems. There is surely enough here to keep many physiologists occupied for many years to come. The work done has added new facts and some new speculations but the central problem has not been solved - indeed it may not be for a long time. It follows, therefore, that there can be no neat ending to this thesis but rather an

indication of where we ought to go in search of further facts.

The question of species difference must be referred to again. Such an explanation of differences is a confession of failure just as unsatisfying to a scientific person as a diagnosis of pyrexia of unknown origin is to the physician. There are in this thesis investigations into the uterine movements of six mammals. This is not mere sordid repetition but it is to be regarded in part as an investigation into species differences in a search - not completely unrewarded - for the fundamental pattern of uterine behaviour. The discrepancies between man and the lower animals are the greatest of all - and this must be a source of great disappointment to the obstetrician and gynaecologist who often point out that our ability to treat successfully human patients has advanced relatively little in comparison with the great increase in our knowledge of the chemistry and physiology of reproductive functions. Any attempt to investigate the human subject meets so many considerations of humanity or of legality that the growth of knowledge must be slow. The methods employed here have received the approval of Corner (1923) who in a description of some of the species differences in reproductive physiology with special reference to oestrus, said: "It is clear that the solution of the cycle of any one species must depend in part upon the progress of knowledge of them all, and that a complicated or obscure cycle like that of the human species will be

intelligible only in the light of simpler types".

But there are other difficulties. There are often grave discrepancies in the reports of different observers working on the same animal. The most notable example of this is the claim made by Knaus (1934) that shortly after ovulation in the human female the uterus became quiet and showed no reaction to oxytocin; this indication of the time of ovulation is the theoretical basis of his "safe period". Of course, whether the period is safe or not depends on quite different observations and he has made most ingenious explanations of any failures that have been reported to him. Very few experimental gynaecologists have been able to confirm Knaus's observations of the behaviour of the human uterus; one is almost driven to conclude that Knaus was (undoubtedly unconsciously) striving to fit in his human work with his earlier experience with the rabbit. Reynolds is a great champion of Knaus, but Reynolds is also a rabbit physiologist and indeed in one place (Reynolds, 1939, p.320 et seq.) he actually says that we ought to be able to predict human uterine behaviour from our knowledge of the rabbit. To my mind the rabbit is one of the most exceptional animals in the whole of reproductive physiology. Reynolds says that other investigators have not followed exactly the Knaus technique and have, therefore, failed to get the "correct" results. There is no imputation of dishonesty in all this but rather the suggestion that investigators

often forget, or perhaps never consider, that by their very investigations they alter the conditions. There is a principle of uncertainty in modern physics which is beyond the mathematical understanding of an ordinary physiologist - but as far as the mathematicians have been able to translate it into ordinary language for my benefit it means that if one makes an exact measurement of, say, velocity then one's measurement of mass at the same instant is necessarily rather inaccurate. (It should be noted at once that this principle of indeterminism is not at all at variance with the principle of scientific determinism as enunciated by Claude Bernard). I only wish that more physiologists would ask themselves what would happen if they had not done what they have done. The very act of carrying out observations necessarily produces some departure from normality. The reasons for this are so obvious that they are often forgotten - anaesthesia, temperature variations, and other climatic alterations, sudden injections of hormones meant to imitate the gradual outpouring of the secretion from its natural factory, the tension on orgars produced by recording apparatus, and so on. Some of these limitations we have got to accept for legal, or humanitarian, or other reasons but occasionally it is possible to go near the ideal. One of the chapters will describe what is, I believe, the nearest approach to the physiological investigation of uterine movements yet undertaken - the movements of the unloaded uterus.

Paradoxically, these experiments involved so much money, so much apparatus and time and trouble that they can seldom be repeated. But this work has reversed the accepted opinion of many years and removed one of the greatest anomalies between the rabbit and other species - the quiescence of the rabbit uterus under the influence of the corpus luteum. The notion that the rabbit uterus was quiet during early pregnancy and pseudopregnancy was suggested by the early work of Knaus (carried out about 1927) and confirmed by Reynolds (1930) by experiments done on the living animal. This is a most important argument in the theory of the maintenance of pregnancy (Reynolds, 1939) and it affords an apparently sound basis for the use of progesterone in habitual or threatened abortion. The spontaneous movements of the uteri of all the other animals examined by me and of the uterus of the human subject (according to most observers) are not affected by progesterone. By this investigation a theory is upset but a species difference of the greatest importance has been abolished - but perhaps as regards the text books one must use the future tense. This new information is, unfortunately, most damaging to the previous simple and satisfying theory.

The rabbit is a conveniently sized animal; it is not expensive in time of peace; it is docile; it reacts to many hormones in the most striking way; the only part of its anatomy not of any great value to physiologists interested in reproduction is

its vagina. But the striking endometrial changes, the more or less permanent oestrus, and the faculty of ovulation after copulation make it certainly the oddest freak in the whole of reproductive physiology. As far as human physiology is concerned it is a snare and a delusion. This diatribe must not be taken to imply that the rabbit is not a useful laboratory animal - much of the work on the corpus luteum hormone would have been almost impossible without it; moreover it has been of great value in elucidating pituitary-gonadal relationships.

There is an often quoted (or misquoted) remark by Kelvin on the value of measuring things. The early investigators of uterine activity made no measurements of activity; it was the introduction of quantitative methods by Robson about 1930 which makes his contribution so valuable. These methods have been applied and extended by the present writer so that statistical methods could be applied. One of the most important features of the method is the measurement of the reactivity of a tissue by finding the smallest amount of a drug which will produce a small but just recognisable change in its activity - i.e. the threshold dose. This method has been used most successfully in the investigation of the special senses where it has produced - considering the difficulties - remarkably constant results. The great advantages of working with small doses are that the effect of a dose passes off relatively quickly and that other organs in the body

are much less affected.

It is many years since Claude Bernard (1865) pointed out the importance of the "milieu intérieure" but it is surprising how many investigations of uterine activity (as well as all manner of other things) have been carried out with surviving organs in an artificial medium. Bernard was interested chiefly in the constancy of the internal environment - a subject recently elaborated by Barcroft (1934). It is but a small extension of Bernard's ideas to suppose that no simple artificial medium can take the place of the 'internal medium' (to use Michael Foster's translation of 'milieu interne'). The writer was recently rebuked by a referee of a well known journal for drawing conclusions from experiments made in vivo because work done in vitro could be so much more exactly controlled. The referee may have been sheltering under the cloak of anonymity but to do him justice I should say that in response to my indignant objections to his view he afterwards accepted the paper. The referee's objections to in vivo work could quite well be substantiated up to a point but they seem to me to be quite unsound for although results obtained with isolated organs may be interesting enough pharmacologically they ignore, and even deny, the importance of the normal blood supply for delivery and removal of metabolites, the normal nervous connections, and the chemical and mechanical relationships with other organs in the body.

The foregoing is then an indication of the philosophical attitude which has developed in the course of the years. One's attitude to life is, of course, chiefly developed by one's experiences - the underdog tends to be a communist, the topdog a conservative. There is a distinct danger of becoming smug even in a scientific subject. I have the satisfaction of knowing that my present Chief, Professor Cathcart, has, presumably on the basis of his experience in nutrition, always inclined to experiments on the whole animal rather than on its disintegrated parts, declaring that the whole is greater than the sum of the parts. He is also very reluctant to transfer results obtained on lower animals to man - especially in the realm of vitamins and mineral metabolism; this heterodoxy is now becoming more sympathetically received. This may be interpreted as an expression of loyalty to the Glasgow School but it is also an indication of the growing pains which seem to accompany the growth of knowledge in any department of physiology.

There is one peculiar defect of the present work which may not be obvious at first sight. The nervous system has been given scant attention - but at least it has been left intact. The process of parturition can be carried out independently of the central nervous system, and therefore, with good reason most of the work has been done on this assumption. But that does not mean that under ordinary normal circumstances the nervous system

does not play a part. Even the ancients knew that the mind had an effect on the uterus - "when she heard the tidings that her father-in-law and her husband were dead, she bowed herself and travailed; for her pains came upon her." (I. Samuel, 4, 19) The physiologist can scarcely be expected to investigate the psychology of labour even if the time were ripe for it, which I very much doubt. There is very little information available about the nervous connections of the uterus beyond pretty drawings of the ganglion cells and a few scattered bits about the stimulation of its nerves; although the reflex contraction on suckling has long been known and has been satisfactorily recorded in women, investigators have failed in the lower animals to find any trace of it. The tracing of pathways through the central nervous system has been easiest where motor activities have been involved, that is where there is a readily recognised end result. The autonomic connections to the heart were early recognised because of the easily recorded changes of heart rate or blood pressure; the connections of the bladder and then of the bowel were discovered much later; the same problem will sooner or later be solved in regard to the uterus.

The troubles that beset the reproductive physiologist do not end here because it has been shown that light and even changes of climate can affect profoundly the oestrous activity (Marshall, 1937). But when the full story of parturition is laid

bare it will be necessary to fit all these rather imponderable influences into it. I am inclined to regard the problem of parturition as only one of the great fundamental problems of the origin of rhythm in the animal body. It is not too difficult to see a partial solution at least of rhythms such as cardiac activity, and even uterine activity on the basis perhaps of what the physicists (after Van der Pol) call a relaxation oscillation. But the cause of rhythms of longer duration - menstruation and parturition - must be expected to be more difficult to elucidate.

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(See also, Olmsted, J.M.D. (1939). Claude Bernard, Physiologist. Cassell. Chapter Twenty).

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(For convenience the references will be given at the end of each Chapter instead of making a complete and probably unwieldy list for the whole thesis. Each reference has been seen either recently or at the time of writing the original paper for publication).

CHAPTER I.

"L'homme de recher ches entraîné à la poursuite d'un problème particulier n'a pas à se préoccuper, autant que dure son effort, du problème général de la science. Ses investigations se concentrent sur un point limité; et pendant qu'il s'occupe à sa tâche dans un coin de l'édifice que la science contemporaine élève avec tant de rapidité, il n'est pas nécessaire qu'il embrasse le plan de cet édifice auquel collaborent tant d'autres études que les siennes. Cependant c'est à realiser ce plan qu'il travaille d'un manière consciente ou inconsciente, comme maçon ou comme architecte".

Claude Bernard (1878).
Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux.
Paris, Bailliere. p. 391.

(Quoted by Olmsted, 1939).

Chapter One

HISTORICAL INTRODUCTION

B.C. 500 to A.D. 1935.

Some of our knowledge of reproduction is very ancient; our prehistoric forefathers must have had some elementary acquaintance with it from their human experiences, and their knowledge would be increased when they began to keep flocks. The scientific attack on reproductive physiology may be said to have begun with the classic experiments of Berthold (1849) on castration and transplantation of the testis. Later Heape, Marshall, Loeb and others accumulated information chiefly on observational and histological aspects. The most important landmark in modern times is the discovery of the cyclical alteration in the cellular content of the vagina of the guinea pig by Stockard and Papanicolaou in 1917. Then followed the work of Allen and Doisy (1923) which eventually led to the isolation of ovarian hormones. In the meantime P.E.Smith (1927) had shown that hypophysectomy could be carried out in the rat with a low mortality rate. The pioneer work of Evans on pituitary extracts was taken a stage further by Smith and Engle and pituitary physiology became of extraordinary practical value when Aschheim and Zondek announced their pregnancy diagnosis test in 1928. A

great spate of work began about ten years ago and shows no sign of slacking off. It is difficult to realise how quickly the face of physiology has been changed since the work described in this thesis began. The chemical constitution of one or two of the ovarian hormones was then being very tentatively discussed and the pure substances were very precious. Indeed in one of the experiments the hormone "progestin" was actually made in the laboratory from sow corpora lutea cut out of ovaries obtained in the Glasgow abattoir. The name "progesterone" was invented a little later (July, 1935) and a few milligrammes of the pure substance obtained from a commercial firm were counted great treasure. In Robson's Recent Advances in Physiology published in 1934 there was not a single structural formula (Robson,1934a). Since that time the chemists have made great strides and many pure hormones of known structure are readily available. Several of the well known commercial firms have given me large amounts of pure hormones and of highly purified hormones which, if valued at the list prices, would represent a considerable subsidy of this work. It is realised, of course, that such co-operation is of value to the firm as well as to the research worker.

This historical review will be confined to material which seems to have a bearing on the problem of parturition. Reviews of the state of our knowledge have been written from

time to time. For the very old literature (1850 to 1905) Kehrer (1907) is a rich source; also Cushny (1906). A review by Gibbons (1927) is useful; that by Allan & Dodds (1930) is almost indispensable for literature up to that time. Later Robson (1934a) gave an outline of the progress made in the early part of the revival of interest in reproductive phenomena. Reynolds' comprehensive Physiology of the Uterus published in 1939 covers a very great deal of the ground both old and recent.

In that mountain of scholarship, Needham's Chemical Embryology published in 1931, the history of embryology is dealt with at great length and the history of the growth of ideas on parturition includes this translation from Volume One.

"Hippocrates (B.C.460 to 377) wrote 'I say it is the lack of food which leads to birth ..... When there is no more food for the young one in the egg and it has nothing on which to live it makes violent movements, searches for food, and breaks the membranes. The mother perceiving that the embryo is vigorously moving smashes the shell. ..... In just the same way, when the child is grown big and the mother cannot continue to provide him with enough nourishment, he becomes agitated, breaks through the membranes and incontinently passes out into the external world free from any bonds. In the same way among the beasts and savage animals, birth occurs at a time fixed for each species without overshooting it, for necessarily in each

case there must be a point at which intrauterine nourishment will become inadequate. Those which have least food for the foetus come quickest to birth, and vice versa. That is all I had to say upon the subject". The flaws in this theory are obvious enough but nothing better was to be produced for a very long time.

In Volume Three Needham gives a list of subsequent theories. Fabricius ab Aquapendente (1570) was of the opinion that the increasing weight of the foetus was the cause of labour. Harvey (De Generatione Animalium, 1651) thought that the amniotic fluid failed or was depraved at this time. In 1821 Mende made the interesting suggestion that the cause of birth lay outside the uterus and regarded birth as due to the stimulus of the tenth missed period. In 1854 Sir J.Y.Simpson (see Simpson, 1871) suggested that loosening or decadence of the membranes, or membranes and placenta constituted the determining cause of parturition and this was the result of degeneration of the decidua. In corroboration of this theory he said that separation of the membranes by a catheter or by introducing tepid water brought on labour. In his lectures of 1856 and 1861 (see Simpson, 1871) he declared that the uterine movements were entirely involuntary but could be modified by emotion. He quoted Brachet who had cut the spinal cord above the origin of the ovarian nerves in guinea pigs and concluded from his findings

that the spinal cord was necessary in labour. But Simpson pointed out that these animals died of rupture of the bladder. If in larger animals the urine was drawn off regularly by a catheter uterine contractions came on after division of the cord. Many years before this (i.e. before 1856) he removed the spinal cord in domestic pigs from the first dorsal vertebra downwards a few days before parturition. Some died, but in the others labour came on normally. All the foetuses were born except the last one (presumably because of the loss of abdominal movements). He quoted also a case of Paget's - a woman was paraplegic from the eighth month but delivered herself at full term without assistance. In 1858 Brown-Séquard found that uterine muscle became more irritable as pregnancy progressed and that excess carbon dioxide in the maternal circulation initiated uterine contractions. In 1874 Goltz confirmed the finding that parturition could be carried out independently of the spinal cord; another experiment was reported later - Goltz & Ewald (1896). The carbon dioxide theory having fallen out of favour Beard (quoted by Needham) revived Mende's theory and produced in 1897 a theory of "critical periods"; the ovary seemed to him to be like an alarm clock which went off at the time set for parturition. In 1907 Kruieger & Offergeld wrote a long paper on the course of pregnancy and labour when the spinal cord was divided by accident in human beings and by design in animal experiments.

They concluded that neither brain, medulla nor spinal cord were necessary for reproduction, and that when the spinal cord was cut through or destroyed labour was slow but otherwise normal.

Michael Foster in the 1891 edition of his text book summarised the situation in the dramatic manner of last century. "We may be said to be in the dark as to why the uterus, after remaining for months subject to futile contractions, is suddenly thrown into powerful and efficient action, and within, it may be. a few hours or even less, gets rid of the burden which it has borne with such tolerance for so long a time. None of the hypotheses which have been put forward can be considered as satisfactory - we can only say that labour is the culminating point of a series of events, and must come sooner or later, though its immediate advent may at times be decided by accident; but it would not be profitable to discuss this question here". A perusal of the works on obstetrics of last century and the early part of this one is most disappointing; in some there were no theories at all, blank ignorance was expressed as to the causes of parturition, in others anything up to eleven theories were given only to be described as "not tenable", "extremely unsatisfactory", or not of "universal application". These theories are reviewed by Marshall (1922). The same rude remarks might be made of some of the newer theories with which we shall have to deal, but at least there is now a considerable body of experimental work

as a basis. An ounce of experiment is worth a ton of arm chair speculation.

The Posterior Lobe of the Pituitary

In 1895 Oliver & Schäfer published a paper on "The Physiological Action of Extracts of the Pituitary Body and certain of the Glandular Organs". They tested the effect of glycerine or watery extracts of various organs on the blood pressure. They found that extracts of the pituitary gland were less active in raising blood pressure than extracts of the suprarenal (adrenalin) but that the effect was more prolonged. This was the starting point of a great deal of work. In a paper "On some physiological actions of Ergot" by Dale (1906) he said 'as in the case of the blood vessels the power of the uterine muscle still to respond by normal contraction to an appropriate stimulus, when the sympathetic motor effect has been completely annulled by ergot, is easily demonstrated by giving pituitary extract intravenously! Among the incidental conclusions is 'The pressor principle of the pituitary (infundibular portion) acts on some constituent of the plain muscle fibre other than that which is excited by adrenaline and by impulses reaching sympathetic axon endings! The discovery of the oxytocic action of pituitary extract was thus announced as an incidental conclusion. In 1908 came the most important histological work of Herring, whom Harvey

Cushing has described as the father of pituitary physiology. Herring, in addition to dealing with the comparative anatomy of the gland, described hyaline bodies which seemed to be produced by epithelial nests derived from the pars intermedia; these hyaline bodies appeared to pass into the third ventricle. The illustrations to this paper are still found in the text-books. Cushing & Goetsch (1910) supported Herring's opinion that the hyaline bodies contained the active principle and declared that the cerebrospinal fluid gave some of the reactions of the posterior lobe extract itself. (Incidentally they stated that removal of the whole pituitary is fatal. This is remarkable in view of the author's reputation; and indicates the great advances which have been made). This work was immediately criticised by Carlson & Martin (1911) who could not duplicate the results when unconcentrated cerebrospinal fluid was used - Cushing and Goetsch had concentrated their specimens about 20 times. Herring carried out experimental work in 1915 which supported his view that pituitary secretion passed into the cerebrospinal fluid. He suggested that the hormone of the posterior lobe might contain two fractions, pressor and oxytocic. Cow (1915) showed that injection of extracts of duodenal mucosa increased the oxytocic and pressor effect of the cerebrospinal fluid. Dixon & Halliburton (1916) said that pituitrin injected into the cisterna magna caused a slow rise of blood pressure and produced a secretion

of milk in a lactating animal but they did not mention the uterus. In some of these papers there was no mention of the oxytocic power of the pituitary extract - the great difficulty about the extracts was their variability. However, in 1922, Burn & Dale published a description of their method of standardisation which has been universally accepted. One worker's results can now be compared with another's. The main purpose of the work was to remove the danger to women in labour of the administration of unknown quantities of a very powerful drug. (It should be noted that the method consists in comparing the contractions of a virgin guinea pig uterus suspended in a bath of modified Locke's solution produced by a standard pituitary preparation with those produced by the unknown material. It is just possible that there may be fallacies in this standardisation in vitro of a substance which is to be administered to a living woman, i.e. in vivo, but so far they do not appear to be serious).

More interest was now displayed in the oxytocic properties of various body fluids and tissues. Dixon (1923) and Dixon & Marshall (1924) showed that injection into dogs of extracts of ovaries in the follicular, but not in the corpus luteum, phase increased the oxytocic content of the cerebrospinal fluid, which was tested according to the method of Burn and Dale. They suggested that parturition occurred when the corpus luteum degenerated and the follicular secretion then stimulated the

posterior lobe of the pituitary, the secretion of which in turn stimulated the uterus. This is a most attractive theory but in a short time it became sadly battered. First Trendelenburg (1924) found that Dixon's estimate of the normal pituitrin content of dog's cerebrospinal fluid was 150 to 1,500 times as great as his (i.e. Trendelenburg's). This opinion was confirmed by Blau & Hancher (1926) who found also that intravenous injection of liver, testis, spleen as well as a commercial extract of ovary without oestrin produced an increase in the oxytocic power of the cerebrospinal fluid. Curiously enough, they found that when these substances were injected into bulldogs no rise of oxytocic power was obtained. Most of these workers seem to have accepted Herring's notion that the active principles of the posterior lobe passed into the cerebrospinal fluid and from there into the blood stream. Geiling in a review of the subject in 1926 said that the mode of exit from the pituitary body was by no means decided; the chief difficulty was the want of a specific and delicate test. That difficulty exists to the present day. When Dixon and Marshall's theory was put to what might be described as a direct test by van Dyke & Kraft (1927) no confirmation was forthcoming; these observers found that, although there was a considerable scatter of results, the cerebrospinal fluid of pregnant women and of women in labour had the same oxytocic power. Then a colossal brick was flung into the works when van

Dyke, Bailey & Bucy (1929) announced, after a series of careful experiments, that the oxytocic substance of human cerebrospinal fluid was calcium. They drew attention to the importance of the fluid used in the Burn and Dale test. Their results are a little surprising, and it must have been more than disconcerting to previous workers to have the bottom knocked out of their solemn theories by a little calcium. In 1933 Friedman & Friedman using the rabbit's uterus in vivo could not find any oxytocic material in cerebrospinal fluid; their results indicated that even van Dyke's results were too high, but they regarded van Dyke's explanation of the previously reported oxytocic action of cerebrospinal fluid as being due to Ca as the most plausible; since then, as far as I know, no more work has been done on the cerebrospinal fluid.

In an important paper published in 1928, Kamm, Aldrich, Grote, Rowe & Bugbee gave a history of the question as to whether the posterior lobe contained one or more hormones which was strangely prophetic of a similar controversy begun about 1935 by Riddle at the Physiological Congress held in Russia concerning the number of hormones in the anterior lobe. Kamm and his coworkers described a method for the separation of the pressor and oxytocic fractions which could be applied on a commercial scale. This work was carried out in the laboratories of Parke, Davis and Co., and the two fractions have since been supplied by this firm

under the names Pitressin and Pitocin; the usual names used in physiology (to avoid advertising P.D. & Co.) are vasopressin and oxytocin. While this work has had useful clinical applications - notably in the avoidance of vascular disturbances when an oxytocic effect only is required - it has also been extraordinarily useful in the investigation of uterine activity as will be shown in the following chapters.

There is a peculiarity of the posterior pituitary which has so far defied explanation. Herring in 1915 found that the skate pituitary had oxytocic properties; more recently it has been shown that in tunicates, the forerunners of the vertebrates, the neural gland (corresponding to the posterior pituitary) contains oxytocic, pressor and chromatophore-expanding hormones (see Scharrer, 1941); in man there is a melanophore expanding hormone in the posterior lobe in addition to the pressor and oxytocic hormones - still stranger the posterior lobe of males contains an oxytocic hormone. What the human being does with his melanophore expanding hormone one cannot imagine; and the utriculus masculinus is a mere relic so that there is very little for the oxytocic hormone to act upon. These statements are not gathered together because they are a collection of anomalies but because they suggest that there is here evidence of some fundamental evolutionary plan. This is a species similarity which we find difficulty in explaining - later we shall encounter species differences which are

equally baffling.

A great blow seemed to be delivered to the oxytocin theory of parturition when Smith in 1931 showed that the posterior lobe was not essential for parturition in rats, and when Allan & Wiles (1932) showed that cats could deliver their offspring normally in the absence of the posterior lobe. Houssay (1935) showed that this statement applied also to the bitch, thus confirming one case reported in 1923 by Dott. Heller & Holtz (1932) who were working in Dixon's laboratory and were therefore more or less committed to his oxytocin theory declared that this objection to the theory seemed hardly justified since the loss of pituitrin caused by the hypophysectomy is quickly amended by hypertrophy of the neighbouring tissues - see Atwell & Marinus (1918), Trendelenburg & Sato (1928), Sato (1928) and Geesink & Koester (1929). But all these workers findings are open to the calcium objection mentioned previously. The question as to the source of the oxytocic material after hypophysectomy must still be held to be incompletely answered.

Relation of Ovaries to Pregnancy

In the meantime a considerable amount of literature had accumulated on the subject of the ovaries and the maintenance of pregnancy. The work is, unfortunately, not easy to describe as a continuous story and to complicate matters further it merges with the work on the pituitary previously described. It has been known since 1897 (Beard & Prenant, quoted by Allan & Dodds, 1930) that the corpus luteum is responsible for the suppression of oestrus and ovulation during pregnancy. Fraenckel began in 1903 the publication of a series of papers to show that the corpus luteum was essential for the embedding of a fertilised ovum. Then followed a great deal of contradictory work (reviewed by Parkes, 1928) on the effect of the corpus luteum on the maintenance of pregnancy; opinion was divided on the subject because some found that removal of the corpus luteum did not affect the course of pregnancy while others stated equally definitely that removal caused abortion. There is not much point in going over this ground because it contributes little to the problem before us. From analogy with other systems of the body the early workers could hardly be expected to accept the fact of species difference which is nowadays so prominent a feature of reproductive physiology; but we are forced to accept these facts and say that there are (without going into minutiae) some animals, e.g. the rabbit, in which the removal of the corpus luteum graviditatis

results in abortion, and there are others, e.g. man, in whom removal of the corpus luteum (once pregnancy is established) is not followed by abortion. The question as to whether there was in the placenta an alternative source of supply of luteal hormone had scarcely been formulated in the period of this review.

The two quite definite functions of the corpus luteum in regard to embedding and maintenance of pregnancy were made the basis of tests of the activity of extracts of the corpus luteum. Corner (1928) reviewed the work of Fraenckel, Loeb, Bouin and Ancel and repeated it under more controlled conditions; he confirmed the existence of progestational proliferation of the rabbit's endometrium during the earliest days of pregnancy, this being under the control of the corpus luteum and necessary for normal implantation. The first satisfactory proof of the presence of a hormone of the corpus luteum was furnished by Corner & Allan in 1929 when they injected an alcoholic extract of the corpora lutea of sows into adult doe rabbits immediately after oöphorectomy and obtained a proliferation of the endometrium similar to that observed in pregnancy or pseudopregnancy (progestational proliferation); further this extract allowed the maintenance of pregnancy in rabbits deprived of both ovaries at the 18th. hour of pregnancy (i.e. shortly after ovulation). In the meantime Teel (1926) had produced rather indirect evidence that pregnancy could be prolonged by the continued action of the corpus luteum

hormone. He injected pregnant rats with anterior hypophysial fluid from coitus to term and found that the gestation period was lengthened by two to six days, failure of the birth mechanism being associated with abnormally persistent lutein tissue. Engle & Mermod (1928) implanted pituitaries in rats and found that the gestation period was not prolonged; on the contrary abortion resulted if implantations were begun before the 15th. day of gestation - but no mass of lutein tissue was observed. The abortions were probably due to oestrin produced by ripening follicles (cf. Parkes & Bellerby, 1926). By sterilisation of one ovary by X-rays Parkes (1928) showed that removal of the other ovary containing the corpus luteum of pregnancy produced abortion, this was definite proof that no other part of the ovary produced the luteal hormone. Nelson, Haterius & Pfiffner (1930) were able to prolong pregnancy in the rat up to six days by extracts of the corpus luteum of the sow. In the rabbit pregnancy can be prolonged by producing a new set of corpora lutea a few days before term by injecting pregnancy urine (Snyder, 1934); this suggests further that neither changes in the foetus nor in the placentae nor distension of the uterus are sufficient in themselves to account for parturition in the rabbit.

At the same time the effect of oestrin (or follicular hormone) on the course of pregnancy was tried out on several

species. The results of the early work have to be taken with a certain reserve for samples of "oestrin" then contained a small amount of protein. Parkes & Bellerby (1926) injected mice at various stages of pregnancy and found that pregnancy always came to an end but that greater doses were necessary for this result towards the end of pregnancy. M.G.Smith (1926) also found that in rats more oestrin was required as the pregnancy advanced. Loeb & Kountz (1928) repeated this experiment on a few guinea pigs without any interruption of pregnancy but they did not specify the dosage used. Other experimenters reported variable results. Reynolds & Firor (1933) injected oestrin (theelin) into two rabbits, one on the 27th. and the other on the 28th. day of pregnancy. Both rabbits aborted and died.

Pituitrin at end of pregnancy

In the meantime Knaus (1926) made an important report on the effect of pituitrin injections into pregnant rabbits. When he injected about 0.01 o.u. (oxytocic unit) of pituitrin intravenously at the very end of pregnancy (31st. and 32nd. days) the rabbit showed straining movements and the first of the young was cast within one to three minutes; when after an interval of 4 to 6 hours the same minute dose was given a second foetus was born, and so on. The pituitary action was thus very slight and was exhausted with the delivery of a single foetus.

When the dose was increased about 10 or 20 times one foetus after another was cast and within 5 to 10 minutes the whole litter was born. At a spontaneous normal birth the young rabbit is born enclosed in the foetal membranes and placenta; under the action of pituitrin the young (especially the first one) were often born without the placenta which followed a little later - sometimes as the result of a second injection.

Increasing doses were necessary up to the 29th.day of pregnancy. Earlier than the 29th.day doses of about 1 o.u. did not bring about abortion but foetal death due to the destruction of the placenta by its separation from the uterine wall. Foetal death was followed later, of course, by abortion. There must be some change in the uterus occurring between the 28th. and 29th.days to account for the alteration in reaction. In support of his contention that his experiments had imitated the normal method of parturition Knaus produced evidence to discount the importance of other factors. Distension of the uterus as a factor in the termination of pregnancy is difficult to assess; in man the duration of pregnancy remains constant in spite of great variations in size and weight of the foetus, in spite even of twins and hydramnios; in full time extrauterine pregnancy the patient has pains at term in the normal way. The important demonstration by Knaus is that one can bring about a very nearly normal delivery by suitable choice of the dose of

pituitrin; it may be, of course, that the same result could have been achieved by the use of another oxytocic agent. Pituitrin is the only substance, however, which is found in the body and which has a good claim to the honour of initiating parturition; histamine is hardly a rival. The fact that we can "imitate" parturition by oxytocin is no proof that it is the oxytocic agent; but it is highly suggestive. One would like very much to know what would have happened if the drug had been infused at a constant slow rate - that would have been (one would imagine) a more exact imitation of a physiological process. One would also like to know what part, if any, the cervix played in the alteration of the behaviour of the uterus on the 28th. and 29th.days - the foetuses may have been retained because the cervix did not relax. This is a matter of considerable interest. An important point to note is that this most significant work of Knaus's was carried out on intact unaesthetized animals. His later work was on the rabbit uterus in vitro - a retrograde step in my opinion.

Oestrin and Oxytocin and Parturition

Miura (1926) showed that after preliminary injection of an animal with placental, ovarian or corpus luteum lipoids the sensitivity of the uterus (when tested in vitro) to oxytocin was very greatly increased. Bourne and Burn (1928a) suggested that since oestrus occurs in many animals immediately after

parturition it seemed likely that oestrin played a part in the mechanism of parturition. In their experiments they used a fairly pure oestrin containing only a trace of protein. They found that oestrin added to a bath containing a surviving uterus increased the effect of a dose of pituitary extract added shortly after, but not the effect of a dose of histamine. The next logical step was to try out in the living animal the effect of oestrin followed by pituitrin. Parkes (1930) remarked that although it had been shown to be possible to hasten or delay parturition by means of ovarian hormones, yet it had never been shown that they could bring about contractions sufficiently vigorous to cause delivery. His experiments showed that (1) oxytocin fails to cause abortion in mice owing to the insensitivity of the pregnant uterus, (2) abortion caused by oestrin alone occurs 36-48 hours after the last injection, but even delayed abortion does not occur regularly, (3) a course of oestrin with a final dose of oxytocin was followed in less than 6 hours by parturition. Marrian and Newton (1932) showed that, in contradistinction to the findings of Bourne and Burn (1928a), when pure oestrin was added to the bath in sufficient dosage an inhibition of the response of the guinea pig's uterus to oxytocin resulted. These findings are, therefore, only of pharmacological interest. This in vitro effect of oestrin was confirmed by Heller and Holtz (1932), Jeffcoate (1932) and Newton (1933).

Bourne and Burn withdrew their previous results in 1932 and stated that they were due to the sensitising effect of the small amount of protein in their original sample of oestrin.

Since Parkes' work in 1930 had been carried out with oestrin containing a trace of impurities it was necessary - in view of the misleading results obtained in vitro - to repeat the work with pure oestrin. In two papers published simultaneously (Robson, 1935a and Marrian & Newton, 1935) the earlier work of Parkes was confirmed. Robson found difficulty in accounting for a number of animals which did not abort under treatment with oestrin and pituitrin; Marrian & Newton regarded the oestrin-oxytocin theory as by no means completely substantiated; because parturition could be brought about by this method it did not necessarily indicate that the body normally employed the same method - the evidence was too circumstantial.

Oestrin-Oxytocin Theory of Parturition

This then is the basis of what has gradually become known as the oestrin-oxytocin theory of parturition; there is in all animals a degeneration of the corpus luteum some time (it may be short or long) before parturition after which the oestrin action goes on unimpeded and later, when the uterus becomes sensitive enough, the oxytocin action occurs. This theory leaves much to be explained but it accommodates easily certain findings regarding oestrin excretion in pregnancy. Aschheim &

Zondek (1927) and Margaret Smith (1927) independently discovered that large quantities of oestrin were excreted by pregnant women. A relatively small amount (500 mouse units M.U. per litre of urine) appeared in the first two months but the amount increased greatly towards the end of pregnancy when sometimes more than 20,000 M.U. per litre were found. (Aschheim & Zondek, 1928a) Runge, Hartmann & Sievers (1932) found that just before parturition the urine might contain more than 100,000 M.U. per litre. The remarkable finding of Cohen, Marrian & Watson (1935) that parturition was accompanied by, and might be preceded by, a rise in the free (as distinct from the conjugated and inactive) oestrin of the urine draws us inevitably to the conclusion that oestrin has an important role in parturition. Jeffcoate (1932) observed that in the Aschheim-Zondek test where the foetus was dead great enlargement of the uteri of the mice used in the test was often seen. This suggests excessive oestrin secretion into the urine, and fits easily into the oestrin-oxytocin theory.

Oxytocic Properties of Blood and Urine

There is a long list of attempts to show that there is an oxytocic substance in the blood and urine of pregnant women at parturition. These are more or less direct attacks on the oxytocic theory of parturition. A great deal of the work has been uncritical and numerous pitfalls have gone unrecognised. Even if blood or urine should show oxytocic properties at term

it does not by any means prove that a substance which stimulates the uterus is being poured into the blood or is escaping into the urine - it may indicate merely a change in ionic concentration or the presence in excess of some quite irrelevant metabolite whose nature has so far escaped us. Both blood and urine, one may be permitted to say, are extraordinarily complex materials, and it would be surprising indeed if they did not possess oxytocic and pressor and other pharmacological properties. But the difference between pharmacology and physiology is like the difference between a toy soldier battle in the nursery and a modern blitzkrieg; the former may be a matter of playing tricks with tissues, the latter is real life. There is one outstanding difficulty in investigating blood; it is well known that it undergoes considerable changes after it is shed. A.J.Clark in some experiments (which I have been unable to trace but to which he directed my attention some years ago) decapitated rats directly above the uterine bath and obtained an oxytocic effect as soon as the blood spurting from the cut carotid arteries mixed with the Locke solution. That the blood of non-pregnant animals should possess oxytocic properties seems in the present state of our knowledge to be a gross anomaly.

It is interesting to note that Brown-Séquard in 1858 injected defibrinated dog's blood containing Co_2 into rabbits near term and produced parturition. Kehrer (1907) showed that urine added

to a bath containing a uterus inhibited the spontaneous movements. Defibrinated blood from women in labour was given intravenously by von der Heide (1911) to women in the last week of pregnancy without effect. He next took foetal blood (i.e. from the cord) after the child was born and found that in some cases the uterus was stimulated; this was regarded as an anaphylactic phenomenon due to the foreign proteins of the foetal serum. This explanation was, of course, a fashionable one because at that time the greatest advances in medicine were being made by bacteriologists and it was natural to look for explanations of all sorts of phenomena in terms of germs or immunity. Brdiczka (1924) found that blood from women in late stages of pregnancy and from parturient women produced strong contractions of the isolated rabbit uterus whereas serum from normal women or from women early in pregnancy had little or no effect; he seemed to be aware of the unspecificity of the test, but he felt that the results were of some importance since they fitted in well with those of Sauerbruch & Heyde (1910) which will be discussed later. A more courageous experiment was carried out by Perez (1930a and 1930b) on a large number of women. He injected 50 to 300 c.c. of citrated blood from women actually in labour into the veins of women at term but not showing signs of labour; this produced increased activity of the uterus in nearly all cases. Blood obtained from women who were in the last month of pregnancy but who were not actually in

labour was ineffective. He reported some reactions which were presumably due to incompatibility of blood groups. Fontes (1929, 1930, 1931) tested out defibrinated blood; to one bath containing a guinea pig uterus he added puerperal blood, and to another similar preparation parturient blood, and saw that the second uterus showed energetic contractions while the first showed very little. By this method he showed that the oxytocic property was not present six hours post partum. Jeffcoate (1932) found, however, that neither maternal nor foetal serum had any effect on the uterus in vitro. According to Englehart (1933) human urine contains a substance which stimulates surviving uteri; the effect varies with the species from which the uterus is taken. The oxytocic material is not padutin, adrenalin, pituitrin, adenosine phosphoric acid, creatinine, uric acid, xanthine or hippuric acid. Cockhill, Miller & Kurzrok (1933) found that urine secreted during labour yielded an extract (obtained by the method of Kamm et al. 1928) which produced contraction of strips of human uterine muscle obtained at Caesarian section at term. The extract had no effect on non-pregnant human uterine muscle strips in vitro. If urine from males or from non-pregnant women was submitted to the same treatment then no oxytocic properties were found. They gave references to the administration of pregnancy urine by proctolysis to test its effect on the uterus. The only pharmacological basis for the work on urine was the early research of

Dale (1909) which indicated that the pressor hormone of the pituitary was excreted in the urine shortly after injection. Dale was then of the opinion that the oxytocic and pressor effects were due to the same substance but now that it is possible to separate these fractions almost completely it would be wise to regard the results of experiments on the oxytocic properties of urine with reserve. Blair-Bell, Datnow & Jeffcoate (1933) injected urine from women at various stages of pregnancy into guinea pigs and later tested the behaviour of the excised uteri. The spontaneous activity and the reaction to pituitrin were both reduced by this treatment; urine from women in labour (but not at any other time) reversed the response to adrenaline; i.e. adrenaline added to the bath gave a contraction instead of a relaxation. This adrenaline reversal can also be brought about by treatment with pituitrin (Cow, 1918, and Heller & Holtz, 1932) but it would be going too far to consider this proof that pituitrin is circulating in the blood during labour because the reversal effect is, like many pharmacological tricks, too unspecific. There is a large number of papers on this adrenaline reversal phenomenon but discussion of it would not be profitable.

The Placenta

It was natural to look to the placenta to provide a key to the problem of parturition; it had been known for a long time that the placenta showed degenerative changes towards the

end of pregnancy and it might be supposed that the placenta exerted some inhibitory effect during pregnancy which disappeared at full term or, alternatively, that at term the uterus was stimulated by some placental secretion so that parturition took place. This speculation was early put to the test of experiment. Blair-Bell & Hick (1909) found that placental extracts stimulated the uterus. Extracts of placenta and ovary were found by Fellner (1913) to have oxytocic properties as indicated by the isolated guinea pig uterus; it is difficult to fit this into any theory of parturition and he realised that the test was unspecific. Brdiczka (1924) expressed fluid from placentae and found that it had oxytocic properties. It was shown by Schumacher (1929) that the administration of placenta either by feeding or by implantation in pregnant mice did not shorten or lengthen pregnancy. Early placental extracts did not cause any inhibitory effect on uterine movements produced either by late placental extracts or by pituitrin. Robson & Illingworth (1931) found that lipoid extracts of full term placentae occasionally caused inhibition of the reaction of the uterus to oxytocin when these extracts were injected into ovariectomised rabbits; earlier placentae did not yield any extracts having inhibitory properties. Similarly Clauberg, Thiel & Ziecker (1932) found it was not possible to get corpus luteum tests on human tissue apart from a large bulk of corpora lutea. Although the search for oxytocic and inhibitory

materials in the placenta has yielded rather contradictory results there is no doubt that the placenta contains powerful pharmacological materials. In 1934 Robson demonstrated to the writer the effects of saline extracts of rabbit full time placentae which had in vitro very remarkable oxytocic properties; a small quantity injected intravenously into a rabbit produced sudden death. These findings have not been published. Snyder (1934) as a result of his experiments on prolongation of pregnancy referred to earlier suggested that the degeneration of the placenta by itself was not sufficient to produce parturition.

The experiments which seem to be most nearly physiological were those carried out by Newton (1935). He found that he could destroy mice foetuses between the 12th. and 15th.day of pregnancy by squeezing them through the mother's abdominal wall without a laparatomy. The placentae continued to live and were delivered at full term about the 20th.day; oestrus occurred one to two days after parturition as is usual after a normal delivery. He called the delivery of the placentae at the normal term "pseudoparturition". There is thus no doubt that in mice the placenta plays an important part in the maintenance of pregnancy and quite possibly in the act of parturition - the exact part is not revealed by these experiments. Haterius (1935) working with rats which, as is well known, abort after removal of both ovaries found that if all but one of the foetuses were removed leaving all

the placentae in the uterine horns the single foetus went on to term but was not expelled. No reason for the failure of the birth mechanism could be brought forward - it certainly is astonishing in view of the similarity of most of the reactions of the rat and the mouse. Newton's experiments seem to be in direct contrast to those of Hammond (1917) which indicated that in the rabbit at least the foetus was of more importance in the maintenance of pregnancy than the placentae. These experiments will be referred to later.

PARABIOSIS

The operation of parabiosis would seem to offer, on theoretical grounds at least, a solution to many of the problems of endocrinology. An acute version of this preparation has been greatly used by Heymans in his investigations of the functions of the carotid sinus and it has yielded remarkable results. The chronic preparation intended to live for several months at least seems to be a matter presenting much greater technical difficulty. The case of the pygopagous Blažek sisters, which has been described as one of nature's experiments, first called attention to the possibility of this experimental method. (Blažek is the correct Czech spelling of the name, which before this spelling was recognised after the last war, was given various German versions). This case was described by Basch (1910). These two sisters possessed one anus, one clitoris, but separate vaginae and internal genitalia; the sisters menstruated simultaneously - the flow lasting from four to five days. This is a freak of the greatest rarity (see Mudaliar, 1930). One sister, Rosa, became pregnant and menstruation ceased; there was little or no disturbance of the other sister, Josefa, who continued to menstruate for seven months. During the pregnancy, Rosa felt the child's movements and was often depressed but Josefa was quite undisturbed. The breasts of both sisters enlarged during the pregnancy and

both secreted microscopically similar milk after parturition. This was taken by Basch to substantiate the view, formed on the basis of his earlier work on mammary transplantation in dogs, of the importance of the placenta as a source of internal secretion in pregnancy. The connection between the sisters was such that materials from the ovaries and placenta of the pregnant sister could travel only by way of the blood or lymph streams. Sauerbruch & Heyde (1910) joined together pairs of rats by coelio-anastomosis; when a non-pregnant animal was joined to a pregnant animal the former had no effect on the pregnancy but at the time of parturition the non-pregnant partner was liable to become very ill unless, as in the case of the Blažek sisters, the parabiosis had been established for a considerable time. This suggested that some toxic material was set free into the circulation at parturition. When two pregnant animals were joined together then the more advanced partner was delivered at the normal time but in three out of five cases the less advanced partner aborted shortly after. This suggested again that there was some material released at parturition which might be responsible for stimulating the second uterus. In 1926 Kross repeated Sauerbruch's experiments but was unable to confirm his results because out of nine pairs of successful double pregnancies in rats only one showed that parturition had an influence on the less advanced partner; unfortunately no statement was made as to

the duration of the pregnancies in each animal - presumably this was not known. Fels in a very wordy article in 1929 discourses on the very great difficulty of making a parabiosis between two pregnant animals; he could report on only one successful case of his own. The first animal was delivered four and the partner nine days after the parabiotic operation. It seems fair to regard the result of such experiments as still in doubt. The care of the animals after parabiosis must be very troublesome and the nutritional requirements for pregnancy were not so well understood then as they are now; it would be worth repeating this work under modern conditions.

Bile Salts

When the inner history of parturition begins to be revealed there will be many little scraps of information to be explained; it is, therefore, worth while to record odd crumbs of information either in the hope that they may offer a clue or because they may be used to test any future theory. Kleesattel (1924) reported that at the time of delivery there was a greatly increased excretion of bile salts into the urine as judged by Hay's test. There was also a rise in the excretion at the puerperium which suggested that the bile salts were more concerned with fat metabolism and milk production than with parturition. Hofbauer (1928) found that bile salts added to the bath suppressed the spontaneous contractions and reduced the response of the

isolated guinea pig uterus to pituitrin, but that the effect of bile salts was less if the uterus was taken from a guinea pig well advanced in pregnancy. There does not seem to be any possibility of building up a theory of parturition on the action of bile salts.

The Cervix

A much neglected portion of the genital tract is the cervix; it is usually and probably erroneously regarded as a rather passive part of the uterus. It might be suggested that delay in labour is occasionally due to an undilated cervix; there may be some unknown factor for co-ordinating the degree of dilatation of the cervix with the development of the labour pains. Kehrer (1907) recorded cervical movements in vitro. Newton's work on the cervix (also in vitro) was carried out later than the period of this review and will have to be discussed with my own cervical experiments. Practically nothing has been done on the activity of the cervix in vivo. Moir (1934) published a tracing of the movements of the body and cervix recorded simultaneously. He found that in most tracings the cervical rhythm was independent of the fundal rhythm - in a few the two were co-ordinated, the cervix lagging about 17 second behind the fundus. He does not seem to have pursued the matter further.

The Foetus

It is more than likely that the foetus itself produces some hormonal substance which plays a part in the maintenance of pregnancy, or in its termination; but the evidence is difficult to obtain and more difficult to interpret. Heape in 1890 transplanted ova obtained from an albino rabbit two days after ovulation into another doe - a Beligan hare - which had been mated to a buck of its own breed three hours before; the young produced, which consisted of two albinos and four Belgian hares, were all born on the 32nd.day after coitus but the albinos were stronger than the others in spite of the transplantation. It would appear that the younger set of embryos determined the time of birth but owing to the mixture of embryos the results are not conclusive. It is curious that this experiment does not appear to have been repeated - it is discussed by Hammond in 1925 as if it were unique. There must be a limit, set presumably by the time of embedding in the uterine wall, to the time difference which could be used but more experiments would be interesting. Hammond (1917) removed the foetuses from pregnant rabbits and left the placentae behind. There followed a regression of the changes characteristic of pregnancy in the ovaries and mammae similar to that found after removal of both placentae and foetuses. This is difficult to reconcile with the experiments of Newton (1935) which have already been described. He found that in the mouse

the placentae rather than the foetuses were the most important factor in the maintenance of pregnancy. Many people must have had a vague presentiment that the foetus itself must play some part in determining when it shall be expelled. Gibbons (1934) rather courageously put forward this notion. He pointed out that there was no doubt that the suprarenals and thyroid are functioning at birth, also the liver of the child is of great size; there is thus no reason to doubt the ability of the child to produce hormones; further after the birth of the child the uterine contractions cease. (This last remark makes one wonder what expels the placenta - one is hardly amazed that the uterus rests after childbirth). Even the latest reviews available in 1941 do nothing more than hint at the possibility of a rôle in parturition (other than mechanical) for the foetus.

Hammond in an important review in 1925 of work on reproduction in what may be termed the "prehormonal age" said that the main results of the statistical investigations into the length of pregnancy in the rabbit from various view points could be summarised as follows. The duration of pregnancy appears to be little affected by the actual bulk of the intrauterine contents but rather by the stage of growth reached by the foetus itself which probably acts through its internally secreting functions on other organs causing birth. (At this time there was no technique - cf. Snyder, 1934 - which would allow of

dissociating the "normal time correlation that exists between the development of the embryos and the corpus luteum of the follicle from which it arose"). This notion of the causation of parturition is important in the interpretation of the experiments of Heape (1890) just quoted.

UTERINE MOVEMENTS

Before we arrive finally at an understanding of the process of parturition we shall have to know in considerable detail the factors governing the activity of the uterus. It certainly is trite to say that it is the muscular contractions of the uterus which are in the end responsible for the birth of the child; but the obvious is not always the most thoroughly enquired into. Investigation of uterine movements forms the major part of the original work to be described in this thesis. Numerous references have already been made to the subject of uterine movements in connection with the action of hormones but we must now attempt to discuss the matter in greater detail as far as 1935.

The early work on the uterus was naturally descriptive of what was seen by the investigator and concerned chiefly the organ in situ, no records being taken. The earliest experiments on the excised uterus were carried out in a moist chamber and can hardly have been very satisfactory. Magnus (1904) was the originator of the well known method of suspending the intestine in an artificial medium and recording its movements on a drum. He used the solution devised by Locke about 1900. E.Kehrer (1907) applied this method to the uterus and many of the earlier workers (but few of the recent ones) referred to the method as the Magnus-

Kehrer method. This is a somewhat neglected but nevertheless quite remarkable paper, although it is of the blunderbuss variety - he had found a new method and he exploited it thoroughly. The movements of dog, rabbit, guinea pig and human uteri were recorded and the effects of quite a number of drugs were described. (Blunderbuss papers are not perhaps so sophisticated as those which set out to test out a hypothesis with a well defined method, and in which a fair idea of the answer is available before the work begins; but nine tenths of original physiological papers are of the blunderbuss category, that is the "let's try it and see" category. A sophisticated smoke screen is laid around at the time of writing up for publication; the journalistic presentation is an exceedingly important part of research work). Kehrer began with a review of the work done last century beginning with Brown-Séquard (1858) but unfortunately most of the references are inaccessible. He (i.e. Kehrer) described the worm like movements of the uterus, how contraction waves passed from the ovarian end of the horns to the cervix as a rule and only occasionally in the other direction; according to him these peristaltic movements were first described by F.A. Kehrer (? father) in 1864 . The two horns might contract simultaneously or independently; the movements were greatest at heat. The cervix and vagina showed smaller and less frequent waves. He found that the uteri of pregnant animals showed quick

contractions and slow relaxations with long pauses between the contraction waves; this description fits very well some of my own records made on the pregnant guinea pig about thirty years later. A large number of drugs including adrenaline were tried out by Kehrer and he found that adrenaline made the non-pregnant cat uterus relax and the pregnant cat uterus contract; in the dog adrenaline produced a relaxation of both body and cervix. He compared movements in vitro with movements in vivo and found substantial agreement. In the experiments in vivo the animal was kept in a bath of warm saline; a thread attached to the uterus passed out through the widely opened abdominal cavity to a recording lever. He considered that it was very much more convenient to study the action of drugs in vitro.

One of the earliest and most refreshing papers on the movements of the uterus in situ is that by Milne Murray (1886) "On some of the physiological and therapeutic effects of water at different temperatures, with special reference to obstetrical and gynaecological practice". It is a simple and direct attack on a given problem - how best to stop haemorrhage after parturition - which could still be used as a model for present day investigators. It had been suggested a few years earlier by Emmett on theoretical grounds that injection of hot water into the vagina and uterus would be valuable in cases of post partum haemorrhage. Murray opened a rabbit's abdomen, attached a clamp

to the cervical portion of the uterus; a thread went to a recording lever from a neighbouring point on the uterus. (It must be recalled that we are here in the pre-Pituitrin and almost pre-injection days). Murray's tracings showed quite clearly that hot water at 120°F. poured on the uterus gave a more prolonged spasm than did cold water; hot water also produced a long spasm of the blood vessels to the uterus without any after dilatation whereas cold water caused a temporary spasm followed by an intense reactionary congestion.

Lode (1894) investigated the problem of the migration of the ovum from the ovary into the fallopian tube in rabbits. He injected carbon particles and later Ascaris eggs into the abdominal cavity and on histological examination found them in the tubes. Lode seemed to favour the ciliary mechanism rather than the peristaltic method. The most interesting part of the paper is the introduction where he reviewed the older literature of tubal movements - going as far back as Haller (1788). He quoted Burdach (1828) who ascribed considerable importance to the nervous control of the tubes. Burdach said that abdominal pregnancies were most common in spinsters and widows who were greatly concerned to keep up an appearance of chastity. He ascribed the accident of abdominal pregnancy to the disordered action of the tubes brought about by the disturbed mental condition.

Modern Methods of Recording Uterine Activity

Several workers not interested directly in the uterus but in pharmacology or in neurology have recorded or observed the

movements of the uterus in situ, e.g. Langley (1901) and Dale (1906). An important paper by Cushny (1906) described the movements of the uterus in situ as recorded by his cardiomyograph (used recently by Robson and Schild (1938) for the same purpose). He noted that pregnant cats and pregnant rabbits showed uterine movements. He also tried out, as did Dale at the same time, the action of adrenaline on various uteri. He reviewed the earlier work most of which is not accessible; it is a curious reflexion on the times that the reviews of earlier work by Kehrer in 1907 and Cushny in 1906 do not overlap to more than a slight extent. Blair-Bell and Hick (1909) also investigated uterine movements in vivo in pithed rabbits. They passed a cannula through the cervix and recorded the change of volume of the saline filled uterus. This is very nearly the same method used in my early work on the guinea pig uterus. These workers found that pituitrin contracted the pregnant uterus and that placental extracts were oxytocic.

Sobotta in 1916 said that ciliary action could not account for the transport of the egg along the fallopian tube because cilia were not always present; he suggested that peristalsis of the tube was a much more likely mechanism. The work of Stockard & Papanicolaou (1917) and of Corner (1921), on the cycles of the guinea pig and the domestic pig respectively, made possible timed instead of random observations; after this period

the work can be described as modern. The early twenties saw a spate of papers on the fallopian tubes and the uterus. The credit of being first in the field is usually given by American writers to Blair (1922 and 1923) who found that the rat uterus in vitro had the slowest rhythm at oestrus, the rate increasing to a maximum late in the resting stage. On the other hand Keye (1923) thought that in the sow the major rhythm occurred at oestrus. Soon after several papers on the movements of the Fallopian tubes in other animals appeared - pig, Seckinger (1923); macacus, Seckinger & Corner (1923); human, Seckinger & Snyder (1924); rabbit and man, von Mickulicz-Radecki (1925 and 1926). The work of all except the last mentioned on the rabbit was carried out in vitro. In the case of the rabbit he observed the movements of the tubes by means of a corneal microscope through an abdominal incision; a very comprehensive review of the earlier work is included in his papers. A quaint method was used by Wislocki & Guttmacher (1924); they excised the whole genital tract at the abattoir and later observed the movements when the whole was placed in a bath of saline. One of the most ingenious and versatile workers in this field is Westman who in 1926 observed the movements of the fallopian tube through an abdominal window; in 1929 we find him using a laparoscope in monkeys; later he used X-ray methods of investigating human tubal movements. A good and easily accessible summary of his work is available as a

lecture (1937). He defined the poles of the ovary by injecting droplets of lipiodol under its capsule, and the movements of the tube were followed by means of a contrast medium injected via the uterus. This is not so very different in principle from the method I used later to investigate the movements of the unloaded uterus. This work on the fallopian tubes is not relevant to the problem of parturition (unless there is a kind of pacemaker in the tubes - but the importance of this is doubtful, especially in animals which carry several foetuses in each horn) but it is important from the point of view of methods; more recent work has added very little new in methods except in complication and in sensitivity. Kok (1925) was probably the first to give records of peristaltic waves passing along the tube; Clark, Knaus & Parkes (1926) gave records of peristaltic waves passing along the uterus of the rat in vivo and found that large waves were well conducted down the uterus at oestrus, they noted large contractions in the pregnant uterus in situ. Clark, Knaus & Parkes tied threads to various points on the uterus and recorded the waves photographically. Curiously enough I used photographic methods ten years later when working in Clark's laboratory and was there led to develop photoelectric methods because of the delay in the photographic process; it is not possible to see what one is recording till the end of the experiment when the bromide paper is developed. Knaus (1926a) recorded movements of the

rabbit uterus in situ by attaching a thread to the uterus which was drawn through a slit in a rubber diaphragm tied across a glass cylinder. The abdominal wall was closed around the cylinder which was filled with Locke solution kept warm by radiation from an electric lamp. This should be a satisfactory method. Knaus (1929) recorded the movements of the human uterus by means of a rubber bag introduced through the cervix; the bag was connected to a mercury manometer and the movements recorded by a stylus floating on the mercury. Reynolds first described his rabbit fistula method in 1930; this was an important advance because it allowed records to be made in unanaesthetised animals. He did a very great deal of important work with this preparation. The movements of the uterus were recorded by means of a balloon inserted through the cervix into the uterine horn. The method was apparently rediscovered by Morgan (1933).

The Movements of the Rabbit Uterus, and the effect of Oestrin and Progestin.

Knaus (1927, 1928 and 1930a) was the first to carry out systematic work on the behaviour of the uterus of the pregnant rabbit in vitro; Robson (1932, 1933a and 1933c) followed up this work and made quantitative threshold measurements. The results of these two workers can conveniently be described together. After mating the uterine movements in vitro became less and less (but never entirely disappeared); when pregnancy was nearly at an

end the spontaneous movements became much more marked and reached their greatest extent at parturition. The reactivity to oxytocin declined very markedly soon after coitus and the threshold dosage remained high for the first 20 days or so of pregnancy - at this time even as much as 5 units of oxytocin added to a bath of 100 c.c. Locke solution containing the uterus might not produce any alteration of the movement of the uterus; but at this time a dose of vasopressin might produce a relaxation. It was only in the last ten days or so of pregnancy that the rabbit uterus reacted at all to oxytocin; the threshold dose fell slowly till at parturition a minute dose was sufficient to make the uterus contract. These findings fitted in very well with the earlier (in vivo) experiments of Knaus (1926), and the experiments of Parkes and others already described on the oxytocic theory of parturition. The explanation of the increase in activity and in reactivity at term seemed clear when it was shown by Reynolds (1931) by means of his fistula preparation that the movements of the uterus in vivo in the unanaesthetized animal were increased after injections of oestrin. Robson (1933c) compared the behaviour of uterine strips from the same animal removed before and after oestrin treatment; he found that in the second case the spontaneous activity was greatly increased and that the threshold dose of oxytocin required to produce a contraction might be similar to the value found in experiments

on the parturient uterus in vitro. The alterations in behaviour in early pregnancy were explained by Knaus (1930) who injected extracts of corpus luteum into rabbits (producing progestational proliferation) and found that the reaction of the uterine muscle in vitro to pituitrin was inhibited. He suggested that this would be a more rapid method than that of Corner & Allen (1929) for determining the potency of luteal extracts. Further work on this suggestion by Illingworth & Robson (1932), by Fremery, Luchs, & Tausk (1932) who went so far as to suggest the name Desensin for a second hormone of the corpus luteum, and by Fevold & Hisaw (1932) showed that the test of luteal activity by desensitisation was not reliable. Fevold & Hisaw were also of the opinion that there was another hormone - other than that responsible for the progestational proliferation of the endometrium - produced by the corpus luteum, this hormone being responsible for raising the threshold to oxytocin. They found that uteri from animals treated with pure corporin (progestin) showed pronounced progestational proliferation yet reacted to pituitrin; on the other hand if impure corporin were given no reaction to pituitrin was observed. This is an almost classical example of the kind of clue that has led to the separation of hormones or vitamins and therefore deserves considerable attention. Reynolds & Allen (1932) were of the opinion that the uterine motility-affecting and the proliferating hormone were

identical. They injected theelin when the uterus was under the influence of the corpus luteum or of progestin. (Injection of theelin usually increases the motility as observed by Reynolds' fistula technique). In neither case was motility restored although the progestational proliferation of the endometrium might be considerably reduced. Alcoholic potassium hydroxide destroyed both properties of corpus luteum extracts. Reynolds and Allen were unable to explain the previous workers results but it is to be noted that they were testing luteal effects not by pituitrin but by oestrin so that the two groups of findings may not be incompatible.

The matter of the unknown hormone has not yet been cleared up but since testosterone produces desensitisation of the uterus (Robson, 1937) but very little progestational change it is just possible that some modification of the progesterone molecule is responsible for the peculiar findings just mentioned. Of course impure preparations may contain oestrin; the ratio progesterone/oestrone seems to be of importance in determining the result of treatment. Leaving this difficulty of one or two hormones aside, the maintenance of pregnancy could well be explained in the rabbit by the early action of the corpus luteum; the onset of parturition could be reasonably accounted for by the decline in the activity of the corpus luteum and the action of oestrin followed up at term by the action of oxytocin from the posterior lobe of

the pituitary gland. It is very curious, however, that in spite of the enormous amount of work done on the rabbit the very core of the problem - i.e. the reactivity of the pregnant uterus to oxytocin in the living animal at various stages of pregnancy - has not yet been reported. The motility (but not the reactivity) of the uterus of the pregnant rabbit in vivo has been described by Reynolds & Firor (1933). As before they tested for the presence of luteal hormone by observing the motility before and after injection of oestrin. On this basis they decided that the corpus luteum is effective up to the 26th. day of pregnancy - i.e. nearly term - in rabbits. This is longer than the period of action which was determined by Knaus on the basis of his in vitro work, and which seemed to be confirmed by the histological work of Siegmund (1931) who found that degenerative changes occurred about the time the uterus became once more reactive to oxytocin. It is unfortunate that Reynolds and Firor did not test the reactivity to pitocin in their experiments. A claim by Reynolds (1932a and 1932b) that the anterior pituitary like substance in blood of pregnant women had a stabilising effect on the uterus because it reduced uterine motility in rabbits has not been substantiated and may be due to extraneous factors contained in the extracts. The extracts were, as in all the experiments by Reynolds, tested for their ability to reduce motility induced by oestrin and no tests of pituitrin sensitivity were carried out.

This particular reduction of motility may not be of great importance because Sager & Leonard (1936) showed that the inhibition of motility by pregnancy urine extracts could easily be overcome by oestrin.

Movements of the Human Uterus

Quite a lot of work had been done on the movements of the human uterus up to the end of the period of this review. The first in the field as far as one can trace was Schatz (1872) and nearly all investigators since then have used practically the same apparatus with only very minor modifications. He introduced a bag into the pregnant uterus; the pressure could be varied by means of a reservoir; the variations in the intrauterine pressure were recorded on a drum by a float on a mercury manometer as in the standard method for recording blood pressure in animal experiments. The actual tracings are very similar to those obtained and published fifty years later; another interesting point is that the tracings were reproduced by a photographic method which was then something of a novelty. There was, however, no mention of aseptic or antiseptic precautions. Kehrer (1907) mentions that Heinricius used a condom placed in the uterus to record its movements in 1889. Of course, information of a subjective nature must have been available from bimanual examinations of the uterus which must have been carried out for very many years; but this

kind of information is not readily described or systematised. Rübsamen (1913) described a method of external hysterography based on the principle that a weight laid on a muscle will be raised when the muscle contracts and will sink again when it relaxes. The method was described in greater detail later (Rübsamen, 1920) and is certainly to be commended for its directness and simplicity. His records seem satisfactory. The most interesting finding from our point of view is a discrepancy between in vivo and in vitro results. He pointed out that chloretone or Pituitrin (Parke, Davis & Co.) which contains chloretone may cause a relaxation of a surviving human tube in vitro but the same Pituitrin (i.e. out of the same bottle) will cause a contraction of the human uterus in vivo as recorded by his apparatus. He was of the opinion that in the investigation of impure preparations only the clinical experimental method is to be trusted. This is strangely prophetic of the investigation of the ergot effect investigated by Moir and others not so very long after. It is quite possible that the cause of the discrepancy was not the chloretone but a vasopressin effect in the first instance and a pitocin effect on the pregnant uterus in the second case - but we shall have to deal with this question again when we come to the work on the monkey uterus.

Coming now to modern times and modern asepsis Bourne & Burn (1927) introduced a rubber bag on the end of a rubber tube

into the uterus; this was connected to a reservoir and a mercury manometer which recorded by means of an ink pen on paper. The bag was introduced through the cervix and placed between the membranes and the uterine wall about eight inches above the os. Records of the changes in pressure before, during, and after parturition were given; the effects of various drugs including that of 2 units of pituitrin were demonstrated. The same authors (Bourne & Burn, 1928), shortly after the work of Kamm et al.(1928), showed that the human uterus in labour (in vivo) was stimulated by a small dose of Pitocin;2 oxytocic units (o.u.) subcutaneously or intramuscularly was approximately the threshold dose. Vasopressin (Pitressin) on the other hand, given by the same route was ineffective in doses up to 12.5 units. Unfortunately from the point of view of this discussion these drugs were not given intravenously because this method, by eliminating the uncertainties of absorption, allows of more accurate estimation of the threshold dosage; the enquiry was entered on by Bourne and Burn from the severely practical need for determining the safe dosage for women in labour. The threshold by intravenous injection would probably be about ten times smaller than that found by intramuscular injection; on a blood volume basis (5 litres instead of 100 c.c.) this is of the same order as that found in the rabbit and human uteri in vitro by Robson. The main value of this work from our point of view is that vasopressin (pitressin)

is most unlikely to be the agent responsible for parturition and that the human uterus is brought into line with the uteri of all other animals by being highly sensitive to oxytocin (pitocin) at term; it also justifies the use of pitocin in investigations of the movements of human and subhuman uteri which will be discussed later.

The Behaviour of the Pregnant and Non-Pregnant Human Uterus in vivo

The best known investigation of human uterine movements in vivos at various times of the menstrual cycle is that of Knaus (1929), also given in his monograph (1934); not only is this the best known but it is one of the most controversial pieces of work in endocrinology. Knaus introduced a rubber bag on the end of a long cannula through the cervix. The balloon and the connecting tubing were filled with water and were connected to a reservoir which could be raised or lowered to distend or relax the balloon. The pressure within the system was recorded by a mercury manometer with a float writing on a smoked drum, as in the standard method of recording blood pressure in animal experiments. It is important to note that this method records the variations in pressure and not the variations in volume of the uterus - it is, however, not necessarily an isometric method. The introduction of the balloon and the cannula through the cervix is accompanied by the usual aseptic ritual. This method has been described in some

detail because although it has since been used by so many investigators, the results have not always been in agreement; accordingly the method must be carefully enquired into to see if we can find the cause of the discrepancies. This work has been valuable because it has stimulated many people to study uterine activity in normal and pathological conditions with the uterus in vivo; this method was also the starting point of the discovery of the peculiar action of the liquid extract of ergot taken by the mouth, which led eventually to the isolation of a new ergot derivative - ergometrine. Knaus's results have been supported by a few and damned by many - it seems that, as in the case of the rabbit, the experimental method influences the results considerably. It is important to note that although Knaus adjusted the pressure in the bag to obtain the greatest excursion of the writing point (a common but quite understandable trick of physiologists) the usual value was of the order of 10 to 20 mm. Hg. The size of the bag was such that it may not have filled the uterine cavity when it was distended under this pressure. It seems to me that it would be safer to use a method which would allow the pressure to be borne by the walls of the uterus themselves; a small balloon under very great pressure in a large cavity may fail completely to record movements because the cavity is never less than the capacity of the distended balloon. This is an extreme case but there is no doubt that the capacity of the

uterus and also its extensibility vary throughout the menstrual cycle. (Schultze, 1932). It is interesting to note that Knaus started off by filling the uterus through a syringe with a bland iodised oil and recording the volume changes in this; one of his cases developed salpingitis and he therefore changed to the balloon method. Knaus found that the human uterus showed slight spontaneous activity in the first half of the menstrual cycle when it responded to pituitrin injected intravenously by contracting. After the sixteenth day of the cycle there was no spontaneous activity and either no reaction to pituitrin or a relaxation. The motility and reactivity to pituitrin returned just before the onset of the next menstrual period. This work fitted well - too well perhaps - the rabbit work of Knaus which has already been referred to. It seemed, then, that in the corpus luteum phase the human uterus was quiet and unreactive as in the case of the rabbit. Thus the explanation of the maintenance of pregnancy under the influence of the luteal hormone made everything easy to understand if one conveniently forgot that pregnancy in the human subject could go on in spite of the removal of the corpus luteum graviditatis (Ask-Upmark, 1926 and Parkes, 1928); the obvious alternative source of the luteal hormone, i.e. the placenta, was found lacking by most observers (Clauberg, Thiel & Ziecker, 1932) up to this date and even more recently the evidence for placental secretion of progestin is

rather slender.

Knaus's work was confirmed in general by Wittenbeck (1930) but he disagreed with the rigidity of Knaus's division of the menstrual cycle. Wittenbeck found, for example, that quiescence might begin on the ninth day (reckoned as before from the first day of menstruation) and that even on the twenty second day there might be considerable motility and reaction to oxytocin in spite of the presence of a well developed corpus luteum verified at operation. Even in the presence of regular menstrual cycles inflammation of the adnexa inhibits the contractions. (In the rabbit on the other hand Laufer & Reynolds (1938) described increase of movements in the presence of inflammation). Wittenbeck described three cases in which he investigated the movements of the human uterus in early pregnancy (Hermstein, Wittenbeck and Tachezy are the only authors I can find who have described movements in early pregnancy); the pregnancy had to be interrupted for some reason in all three so that the apparatus could be inserted into the uterus without respect for its contents. In two women, one two months and the other three months pregnant, the uteri showed spontaneous movements and reacted to oxytocin; in yet another case 3-4 months pregnant no movements were seen and no reaction to oxytocin was obtained. The first two cases show that in the presence of well developed corpora lutea the pregnant uterus may be active and reactive.

A little later Hermstein (1931) reported four cases of early pregnancies in women (2-3 months) in whom the uterus did not react to pituitrin. In each of these medical abortion was indicated. He also found difficulty in accepting Knaus's rigid division of the menstrual cycle; in women with 3-week periods he found no reaction to pituitrin at the 10th.day; women with 4-week cycles conformed generally to Knaus's picture but one of these cases showed no reaction at the 9th. day and another no reaction on the 5th.day of the menstrual cycle. Five women, amenorrhoeic for $4\frac{1}{2}$ months up to $1\frac{1}{2}$ years, showed no uterine activity and no reaction to pituitrin; obviously this was not due to the presence of a corpus luteum but to some other endocrine or nervous disorder. He thought that Knaus's method gave a good indication of the presence of follicles (by uterine motility and reactivity to oxytocin) but did not necessarily indicate the presence of corpora lutea (by quiescence and lack of response to pituitrin).

Schultze (1931) investigated very carefully the effect of pituitary extracts on the uterus. He filled the uterus with a radio-opaque medium and observed the contour of the uterus and the pressure within it at the same time by means of a spring manometer attached to the tubing near the syringe. The manometer was read at intervals of 6 seconds and the curves drawn later. He obtained stronger reactions to pituitrin as the menstrual cycle advanced - early in the cycle he obtained weak (or no)

responses to pituitrin. His pressure base line varied from 10 to 60 mm. Hg, but several of the published curves were within the range recommended by Knaus. He regarded the difference between his own and Knaus's work as due to technical differences; because Knaus did not observe the filling by X-rays he could not say when the uterus was full - this was particularly the case late in the menstrual cycle when the uterine cavity was largest (Schultze, 1932). It seems to me that Schultze's objections apply with even greater force when a small balloon is used (e.g. by Knaus) in the later stages of the menstrual cycle.

Knaus's work has also been criticised by Tachezy (1934) and Moir (1934). Tachezy does not state the pressure in his balloon so that one can only record his finding that the pituitrin responses are greatest towards the end of the cycle. He investigated five cases of human pregnancy; two were in the first month, and there was one in each of the third, fourth and fifth months. The first two were investigated by Knaus's method and the others by the Tokodynamometer of Crodel (presumably a method of external hysterography similar to that of Rübsamen described above). In all he obtained a positive reaction to one drop of Pituitrin but he published no curves from these pregnant cases. Moir's experiments on the other hand seem to have been carried out as nearly as possible like those of Knaus. Moir found that in the non-pregnant uterus the spontaneous contractions increased towards

the end of menstrual cycle and that pituitrin at all times produced a contraction. A most important observation in this paper was that the non-pregnant uterus never responded to oxytocin but always to vasopressin. He confirmed the earlier demonstration of Bourne and Burn that the parturient uterus reacted to pitocin. This seems to me to indicate that the state of the uterine muscle at the end of pregnancy is quite different to that in the non-pregnant woman; and presumably the hormonal environment is entirely different in the two cases. Theories of the maintenance pregnancy based on the behaviour of the non-pregnant organ are, therefore, liable to very grave fallacies. It may be that investigation of this change from vasopressin sensitivity to oxytocin sensitivity will provide an important key to the problem of parturition and perhaps to the discovery of new hormones. This subject will be re-opened in later chapters.

The Pregnant Human Uterus in vitro

About the same time Robson had been making investigations into the behaviour of various uteri in vitro. In 1933 he published a report of investigations into the behaviour of the human pregnant uterus in vitro (Robson, 1933b). The strips were removed at operation and kept in Ringer at a low temperature till they reached his laboratory from various hospitals in Glasgow and Edinburgh. (The importance of this detail will be seen later when we consider the work on the uterus of the rhesus monkey).

Since the operations were carried out in cases of contracted pelvis, heart disease and so forth, the strips can be regarded as being as nearly normal as possible. The results of this investigation are very important because they are still the only continuous and quantitative investigation available. I shall have occasion to point out later, however, that we cannot transfer the findings without a large qualification to events in vivo. Robson found that early in pregnancy the uterus either did not react at all to oxytocin or reacted only when a very large dose (e.g. one oxytocic unit to a bath of 100 c.c.); at the end of pregnancy the reactivity to oxytocin was very high - the threshold dose might be 0.005 o.u. Thus there is a very striking similarity to the findings in the rabbit. Experiments carried out by Robson (1935b) in which he made a direct comparison between the behaviour of the uterus in vitro and in vivo showed that there was very little discrepancy. The inference was that this statement applied to other animals including the human being.

The work of Knaus and Robson on the human subject and on the rabbit fitted together very well and made the story of the maintenance of pregnancy very simple. It appeared that the corpus luteum was responsible for the low reactivity to oxytocin and that the degeneration of the corpus luteum plus the action of oestrin-formed perhaps in the placenta if not in the ovary -

would account for the high reactivity to oxytocin observed at the end of pregnancy. Allen, Pratt & Doisy (1925), Zondek (1931), and Catchpole & Cole (1934) showed that the placenta contained oestrin while Jeffcoate (1932), Brindeau, Hinglais & Hinglais (1934) and Allan & Dodds (1935) showed that oestrin could be excreted in large quantities at the end of pregnancy in spite of the early removal of the ovaries. (The subject is reviewed briefly in the last named paper). The site of oestrin formation under normal circumstances is thus likely to be chiefly the placenta.

Uterine Movements in other Animals

A few experiments reported during the period of this review did not fit into the accepted theory. Robson (1934) showed that strips of the uterine muscle of the pregnant mouse in vitro showed at no stage complete absence of reaction to oxytocin - he considered that the corpus luteum played little, if any, part in determining uterine reactivity, in the case of this animal. Siegmund (1930a, 1930b and 1930c) and Siegmund & Kammerhuber (1931) studied the uterus of the rat, mouse and guinea pig in vitro under the influence of corpus luteum extract. This work was carried out in the laboratory where Knaus worked. They confirmed the work of Knaus on the rabbit but they could find no effect on these other species although there was no doubt from rabbit tests that the extract of the

corpus luteum which they used was potent. This, then, was the first crack in a very useful theory - but greater gaps were soon to appear; these will be discussed in due course.

This review will inevitably leave the mind of the reader in a haze. But so do all other reviews of this subject - there is always more material than substance, more speculation than fact. This may be the clue to its fascination.

Further advances could only be made in two circumstances - (1) if pure hormones became available, or (2) if new and specific tests were invented for the presence of the hormones. The first of these conditions was soon to be fulfilled but the second has certainly not been fulfilled. As new hormones have been discovered it has been found that their physiological effects overlap to varying degrees. Still more difficult among the oestrogens there is a considerable similarity of effect among very different chemical substances - i.e. the ability to produce oestrus is now known to be a much less specific effect than was earlier imagined. As knowledge accumulates so do the difficulties.

We must now consider the original contributions to this field; finally an attempt will be made to bring up to date the views and conflicting opinions on the causation of parturition.

> "To every thing there is a season, and a time to every purpose under the heaven: a time to be born, and a time to die; a time to plant, and a time to pluck up that which is planted".
>
> Ecclesiastes, III., 1 and 2.

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"Around me I behold,
Where'er these casual eyes are cast,
The mighty minds of old;
My never failing friends are they,
With whom I converse day by day".

Southey.

ORIGINAL WORK

"Originality and independence are the characteristics of a genuine research, and it is stultified by the acceptance of standards and by the recognition of authority".

J. Y. BUCHANAN,

Comptes rendus of Observation and Reasoning, 1917.

Chapter Two

THE OXYTOCIC PROPERTY OF THE BLOOD OF THE COW

From the Hannah Dairy Research Institute, Ayr, and the Institute of Physiology, University of Glasgow - 1934.

This chapter contains the first instalment of the frontal attack - as I termed it earlier - on the problem of parturition. It so happened that S.Morris, who was then on the staff of the Hannah Institute, had been tackling the question of the metabolism of goats at the time of parturition. He could not pursue his work beyond a certain point by ordinary chemical procedures and I was asked to see what could be done to carry the matter a step or two further by biological methods. I took down to Ayr the necessary kymographs and uterine baths and other materials from the Physiology Department. Morris was responsible for the small amount of chemical work involved while I carried out the biological estimations.

The earlier indications that the posterior lobe of the pituitary gland was involved in parturition have already been given in the previous chapter. The work which seemed in 1934 to be most relevant was that of Fontes (1929, 1930 and 1931), Jeffcoate (1932) and Heller & Holtz (1932). Küstner & Biehle (1927) found that blood obtained from pregnant women and especially from women in labour enhanced the power of pituitrin to

cause expansion of the frog's melanophores, whereas the blood from non-pregnant women or from women in the puerperium actually inhibited slightly this action of pituitrin; if one assumes for the moment that the posterior lobe hormones are one and indivisible this would suggest that at parturition there is something in the blood which aids the oxytocic action of the posterior lobe. This problem too could only be tackled by biological methods; it is, of course, a long jump in speculation but the finding like others will have to be fitted in somewhere into an all embracing theory of parturition.

Morris's work (published in 1933) was carried out on goats. He found that from four to six days prior to parturition there was an abnormal protein catabolism which could not be due to an intense acidosis since ketone bodies were not found in the urine. Analysis of the distribution of the nitrogenous end products in the urine suggested that the excess nitrogen excretion arose from the break down of muscular tissue. He suggested that this state of affairs was due to some substance not present in the animal's body except at parturition. Injection of pituitrin into goats produced an increase in N output (if the dose were sufficient) with an alteration in nitrogen partition very similar to that occurring at parturition. The evidence is good, albeit circumstantial, that the posterior lobe hormone is in the circulation at parturition; the next logical step is to

see whether in fact it is to be found by biological methods in the blood stream.

Method.

The only method available for the assay of the oxytocic hormone is the uterus method; any test based on diuresis or melanophores or even blood pressure avoids the real question of the action on the uterus and presumes that all the properties of the extract of the posterior lobe of the pituitary go hand in hand. The excised virgin guinea pig uterus was chosen because it was (and still is) the standard test object for the assay of extracts of the posterior lobe. In view of the fallacies which had already caused so much trouble in the investigation of the pharmacology of oestrin (see Bourne & Burn, 1928 and 1932, and also Marrian & Newton, 1932) it was necessary first to make a protein free filtrate or extract of blood. The fact that much of the work on the oxytocic properties of blood had been done without this precaution made its significance open to serious doubt (e.g. von der Heide, Brdiczka, Perez and Fontes quoted in Chapter One).

There is considerable difficulty in obtaining a protein free filtrate as will be seen in a moment. The possibility of using nature's filtrate, the cerebrospinal fluid, was considered; but the subarachnoid space in the cow is at a considerable distance from the surface. This anatomical difficulty and the fact that we were dealing with a non-co-operating

animal in a pedigree herd worth a substantial some of money ruled out the method. Maclean (1928) dialysed blood through membranes against Ringer solution; this method is slow and it dilutes the blood considerably. Anselmino Hoffman & Kennedy (1932) filtered blood through collodion membranes. This method is only suitable for very small quantities because the filter is rapidly clogged up with the protein of the blood; it was found that after a few c.c.s had come through at a reasonable rate no further filtration would take place; occasionally a little protein leaked through the membrane. This method had also to be abandoned after a short trial as useless. It is easy enough to remove protein chemically for the purposes of chemical estimations (e.g. blood sugar) but since nearly all the precipitants - heavy metals, tungstic acid, etc. - are toxic to biological material they were ruled out without a trial. The method finally adopted was as follows.

Large quantities of blood can be safely taken from cows and therefore the dairy herd of Ayrshire cows in the Institute farm was used. This work owes quite a lot to the co-operation of the farm manager, Mr.A.B.Fowler, who taught me the method of venesection. The cow was tied up in the byre by the usual collar or chain, an assistant held the nose while a strong cord was passed round the neck and kept tight enough to make the external jugular vein distend. Although this vein is almost

unbelievably large to those who have done venesections in the human subject only, it is at the same time very freely mobile; a successful venesection requires a sharp stout needle (and incidentally a stout heart for those, like myself at that time, quite unused to such large animals) and a clean direct thrust into the vein. No attempt was made to take aseptic precautions - it would be impossible to sterilize a cow's hair and skin. 150 c.c. of blood were run into a conical flask containing 5 c.c. of 5 p.c. sodium citrate. This blood was then centrifuged to bring down the red cells, the plasma was pipetted off and 1 c.c. of concentrated hydrochloric acid (36.6 p.c. HCl) was added to every 10 c.c. of plasma. The acid was added slowly with constant rotation of the flask to prevent the formation of a thick gelatinous mass. The protein precipitated at this stage was centrifuged off. The supernatant fluid was decanted, raised to the boiling point and quickly cooled; this precipitated any protein remaining in the acid solution. After a final centrifuging the supernatant fluid was decanted off and kept on ice till required. It is to be noted that this stage by stage removal of protein is necessitated by the large amount of protein in blood - the red cells contain about 30 p.c. of protein and the plasma about 8 p.c. of protein. If the acidifying and boiling are carried out without intermediate centrifuging an unworkable mass like a hard boiled egg is obtained. The procedure may look obvious

enough now but it took a little time to reach this satisfactory method. The final extract gave none of the usual tests for protein (precipitation by heavy metals) but it gave a faint biuret reaction due possibly to traces of metaproteins or peptides. Since, however, the conditions of preparation were kept rigidly constant it was not to be expected that these traces would affect the estimations. The solution was neutralised immediately before it was added to the uterus bath with a 10 p.c. solution of caustic soda - a drop was taken out from time to time to be tested with British Drug Houses Universal Indicator on a porcelain slab. Usually about 0.4 c.c. of NaOH were required for each 1 c.c. of extract. The volume of the final test solution was nearly the same as that of the original blood, as indicated below:-

100 c.c. bovine blood yields 60 c.c. plasma (Blackwood, 1932).
6 c.c. conc. HCl are added.
26.4 c.c. 10 p.c. NaOH are required for neutralisation.
Final volume 92.4 c.c. test solution.

For the calculation of the results it was assumed that the test solution was equal in volume to the blood withdrawn from the vein.

The great advantage of this method was that it introduced no foreign or new ions into the extract - the ions involved being only H^+, OH^-, Na^+, and Cl^-, all of which were present in the blood before treatment. Although the concentration of salts in the final extract differed markedly from the concentration of

salts in the Burn and Dale solution the small amount of extract added to 100 c.c. of this solution should have made very little difference to its osmotic properties. Curiously enough this point was not tested out directly at this time but at a meeting of the Physiological Society in Bristol Sir Henry Dale himself agreed that this would not be likely to be a source of error as almost certainly the uterus would relax rather than contract if the osmotic pressure of the solution rose to any extent. In the preliminary deproteinising experiments blood obtained at the slaughter house was used; when ultrafiltration had been found unreliable acidifying with acetic acid was tried. After boiling the supernatant fluid still contained protein. It appears to be necessary to get the pH below 2.8 before the serum proteins can be completely precipitated. The addition of the stated amount of hydrochloric acid brought the pH well below 2.8, and the final product after boiling was quite clear and protein free.

In the actual estimations the method of Burn & Dale (1922) was followed, but owing to the fact that only small quantities of oxytocic substance were found it was not possible to use submaximal doses which Burn & Dale found to be reliable. The region 1/3 to 2/3 of the full contraction of the uterine horn was used as it has greater differential sensitivity (W. Storm v. Leeuwen, 1923; Sawasaki, 1925) although it may not have

the same stability as the submaximal region. At first the guinea pigs were spayed a few days before the test to ensure that they would not be in heat at the time of the assay; but this method did not produce more stable uteri, that is uteri showing fewer spontaneous contractions than are usually exhibited by unspayed virgin uteri. These spontaneous contractions can be more than exasperating to the experimenter and are rather seldom referred to by those with most experience of the method; Newton (1933) found that if guinea pigs were spayed a considerable time before the uteri were put into the bath they would still respond quite accurately to pituitrin but that they took a very long time to reach the maximum height of the contraction and that this increase in the time of the experiment was a greater disadvantage than the occurrence of spontaneous movements.

Each assay lasted a considerable number of hours and was carried out by adding test solution and standard solution at regular intervals, changing the Burn and Dale solution as soon as the muscular contraction had reached its height. The aim was to adjust the dose of the blood extract put into the bath containing the uterus so that the resulting contraction was the same height as the preceding and following contractions produced by a known volume of the standard solution; or, failing this, to get a contraction height between a smaller and a

bigger produced by known amounts of the standard pituitrin solution. In many cases it was found that the sensitivity of the preparation rose during the assay; this is a general tendency of the isolated guinea pig uterus but it may have been increased by the blood extracts in spite of the fact that the bath was washed out several times between each addition of test solution. The increase in sensitivity was very useful when blood extracts with a very low oxytocic power were being examined; it also meant that each assay was carried out at several different levels of sensitivity on each uterine horn. In the results the potency of the blood extracts is expressed in terms of international oxytocic units per litre, using Pituitrin (Parke, Davis & Co.) diluted to give 20 oxytocic units (o.u.) per litre as the standard of reference. The reaction of the Burn and Dale fluid was always adjusted to pH 7.4 using bromthymol blue as the indicator; oxygen with 5 p.c. carbon dioxide was used for aerating the uterine baths. The results quoted below are the average values given by the assay on two uterine horns - each horn being set up in a separate bath for this purpose.

Results

Heifers. The blood from fourteen virgin heifers was examined. In ten cases there was no sign of oxytocic power - that is each contained less than 1 o.u. (oxytocic unit) per litre which is about the threshold of the method. In four cases the

oxytocic value varied between 1.0 and 3.5 o.u. per litre. The period of heat seemed to make no difference to the oxytocic power of the blood.

Name	Date of Sample	Assay o.u.per litre	Remarks
Kirkhill Greta	10.10.33	<0.8	
Nippy	11.10.33	3.0	
Jessamine	16.10.33	<1.0	
Catherine	16.10.33	<1.0	In heat
Nancy	16.10.33	<1.0	
Kirkhill Delight	17.10.33	<1.0	
Nerissa	17.10.33	<1.0	
Sally	17.10.33	<1.0	
Miss X	18.10.33	<1.0	
Netta	18.10.33	3.0	
Kirkhill Linnet	18.10.33	<1.0	
Kirkhill Hopeful	18.10.33	<1.0	
Giddy	20.10.33	1.3	
Ella	20.10.33	3.5	

Cows. Forty five blood samples were taken from fourteen cows and from two pregnant heifers. (An animal is called a heifer up to the time of delivery of her first calf). Dry and non-pregnant cows, Fig.2 (1), and cows in early pregnancy and in milk showed no measureable oxytocic substance, Fig.1 A.

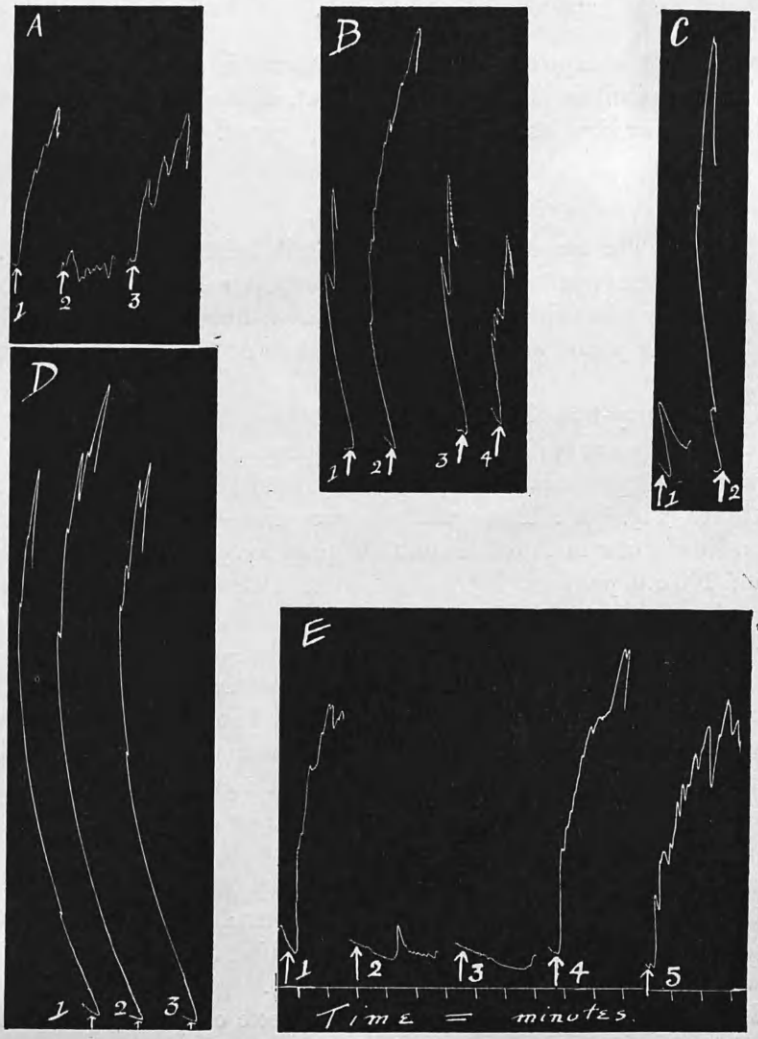

Fig. 1. Test solutions T prepared in the manner described in text. S, standard solution of pituitrin (20 o.u. per litre).

A. "Spring Morn," 14 days *ante partum*: (1) 0·7 c.c. S, (2) 5·0 c.c. T, (3) Repetition of 1.

B. "Kirkhill May Mist," 3 hours before calving: (1) 1·0 c.c. S, (2) 2·0 c.c. T, (3) Repetition of 1, (4) 1·5 c.c. T.

C. "McMinn," 22 hours *post partum*. (1) 5·0 c.c. T, (2) 0·3 c.c. S.

D and E. "Threave May Mist," 5 months pregnant. T_1 prepared in usual way. T_2 pituitrin added to blood to give 20 o.u. per litre. Solution then prepared in usual way.

D. Immediately after preparation of solutions. (1) 0·5 c.c. S, (2) 0·1 c.c. T_1, (3) Repetition of 1.

E. 24 hours later. (1) 1·0 c.c. S, (2) 0·2 c.c. T_1, (3) 0·4 c.c. T_1, (4) Repetition of (1), (5) 1·0 c.c. T_2.

There was one exception - two separate samples taken from a cow in the fifth month of pregnancy had the equivalent of 40 o.u. and 100 o.u. per litre - Fig.1 D. No other animal at any time showed oxytocic values approaching this, so that it must be regarded as exceptional. Measurable quantities - 1 to 18 o.u. per litre - were first noticed at the time of the "drop" or relaxation of the sacrosciatic ligaments. This occurs about one week ante partum in the cow but may occur two or three weeks ante partum in the heifer. This heightened oxytocic value was maintained and increased up to the time of parturition (Fig.1B). In four cases there was a temporary drop in oxytocic content two or three days before calving; the significance of this is doubtful, although it coincides in time with a fall in nitrogen excretion noted by Morris (1933).

Calved cows. Five samples were obtained from normal cows in the first week of the puerperium - that is from seven hours to eight days post partum. None of these showed measurable oxytocic power (Fig.1C).

Name	Served	Delivered	Date of Sample	Assay o.u./litre
Nancy	24.7.33	-	4.8.33	<2
Knockbirnie	5.7.33 and 15.8.33	-	25.7.33 13.10.33	<2 <1
Treeshill	8.7.33		25.7.33	<2
Dunlop	3.5.33	-	24.7.33	<2
Threave May Mist	about 4 months before		9.8.33 17.8.33	100 40
Jessamine	-	22.8.33	11.8.33 16.8.33 21.8.33 22.8.33 7 hours p.partum	25 <4 12 <0.8
Peggy	-	24.9.33	24.8.33 27.9.33	<5 <1
Miss MacMinn	-	1.9.33	25.8.33 27.8.33 29.8.33 1.9.33 2.9.33	2 ("drop") 3 <2 4 <1
Kirkhill May Mist (heifer)	-	19.9.33	25.8.33 29.8.33 4.9.33 18.9.33 18.9.33 27.9.33	<6 1.7 ("drop") 5.5 13 13 <0.6
Hopeful	-	-	30.8.33 17.9.33	<1 <0.6
McNaughton	-	??	31.8.33	3 ("drop")
Ena	-	-	1.9.33	<1.3
Catherine	-	-	1.9.33	<1.3

Name	Served	Delivered	Date of Sample	Assay o.u./litre
Delight	-	9.10.33	6.10.33	<1
			9.10.33 2 hours a. partum	2.7
Joan	-	-	27.7.33	<2
Donella	24.5.33	-	31.7.33	<2
Giddy	30.9.32	15.9.33	31.7.33	<2
			23.8.33	<5
			31.8.33	2.5
			16.9.33	<1
Bountiful	28.12.32	3.10.33	1.8.33	<2
			24.8.33	<6
			8.9.33	<2
Spring Morn	1.12.32	7.9.33	1.8.33	<2
			23.8.33	<3
			31.8.33	2.8
			5.9.33	<1
			7.9.33 1¼ hours a.partum	4.4
Sally	-	18.7.33	2.8.33	<1 (in heat)
Milkpail	-	23.11.32	3.8.33	<2
			30.8.33	1
			5.9.33	<1
			14.9.33	<0.6

It would appear that normally the oxytocic content of the blood is very low - certainly less than 1 o.u.per litre. During the last week of pregnancy the amount becomes measurable - about 5 o.u. per litre. After calving the oxytocic value falls quickly to less than 1 o.u. per litre. When the amount is quoted as being less than a certain value it means that this value was the smallest amount that the guinea pig uteri

used would respond to - this threshold value was usually about 1 o.u. per litre. It would have been inadvisable to use greater quantities of the blood extract to reduce the threshold since this might have led to spurious results by upsetting the osmotic and hydrogen ion concentrations in the uterine bath.

It is important to note that these experiments showed that there was in blood prepared by this method an oxytocic substance near the time of parturition; they could not of course demonstrate anything more than this coincidence. To say that this substance is the cause of labour would not be justified - indeed there is some evidence against it because in several cases the oxytocic content of the blood was high a considerable number of days before calving.

The Nature of the Oxytocic substance

It has been indicated in the historical survey that the guinea pig uterus reacts to very great number of substances by contracting; it is thus a highly unspecific test object. It was, therefore, of considerable interest and importance to see how the oxytocic substance which had been found in the blood of the cow near parturition compared with the oxytocic material of the pituitary; and also to find how the oxytocic property of the blood altered when the animal was injected with posterior lobe hormone. Even if the oxytocic material should prove to be

different from that found in the pituitrin ampoule it does not at once deprive it of any claim for consideration since all that the oxytocic theory demands is that an oxytocic material should be produced at parturition somewhere in the animal.

The first experiments were carried out in vitro. In six cases Pituitrin (Parke, Davis & Co.) was added to cows' blood after removal from the jugular vein. After removing the protein in the manner indicated above there was no loss, within the limits of experimental error, of the added Pituitrin - Fig.1E (5). This showed that Pituitrin could survive the acidifying and boiling and that it did not go into the red cells.

It was found further that citrated blood stored in the ice chest retained its oxytocic power (if present) for at least 24 hours. The acid solution prepared from the blood did not retain its oxytocic power even when stored in the ice chest. The acid solution prepared from blood to which pituitrin had been added did retain its oxytocic property. The oxytocic substance detected near parturition is therefore much more labile than posterior lobe extract - Fig.1, D. and E.

Of the many substances which can produce a contraction of the guinea pig uterus in vitro, histamine is the most important example; others have been referred to in the historical survey in Chapter One. Histamine is produced in the body when tissues are damaged and also in muscular exercise. It was found

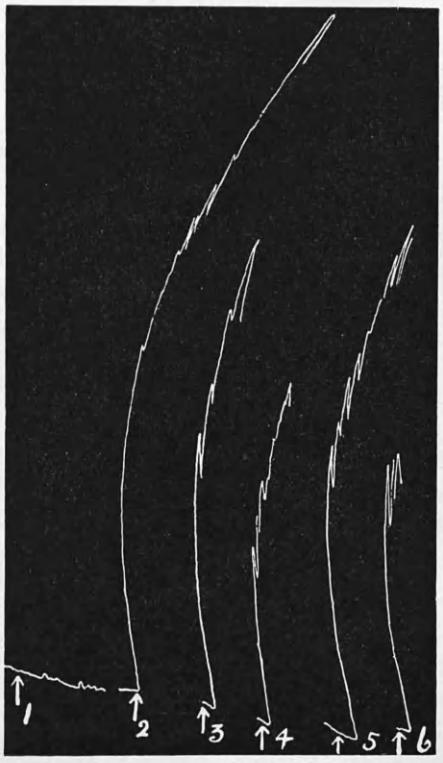

Fig. 2. "Hopeful." Test solutions *T* prepared as described in text. (1) 3·0 c.c. *T* from blood withdrawn immediately before injection of 10 c.c. pituitrin. (2) 0·7 c.c. *T* from blood withdrawn $1\frac{3}{4}$ hours after injection. (3) 0·05 c.c. standard solution of pituitrin (20 o.u. per litre). (4) 0·4 c.c. same *T* as in 2. (5) Repetition of 3. (6) 1·0 c.c. *T* from blood withdrawn $3\frac{3}{4}$ hours after injection.

that on allowing the neutralised or alkalinised extract to stand at room temperature for a few hours all its oxytocic powers disappeared. Histamine can thus be excluded since it is stable in the presence of alkali (Burn, 1928).

Van Dyke, Bailey & Bucy (1929), using the excised uterus method, stated that the oxytocic substance of cerebrospinal fluid was almost certainly calcium. Friedman & Friedman (1933) using the rabbit fistula technique devised by Reynolds (1930) agreed with this opinion. In the present series the greatest oxytocic values were found in late pregnancy and in milk fever (see Chapter Three) where it is known that the serum calcium is lower than normal. Also the fact that oxytocic property disappears on standing and on neutralisation is against the idea that in the present series of experiments calcium was the cause of the uterine contractions.

When neutralised ready for addition to the bath the test solution contained 4.7 p.c. sodium chloride. The hypertonicity did not appear to induce contractions of the uterus since there was never parallelism between the amount of test solution added to the bath and the height of the contraction obtained - see Fig.2, 1 and 4.

The oxytocic power of blood extracts is thus due to some material which is neither posterior lobe extract nor histamine nor calcium; and it is not due simply to an alteration

of ionic concentration.

Three experiments on the nature of the oxytocic substance were carried out in vivo. Large doses of Pituitrin were given by intramuscular injection to non-pregnant cows yielding only a small quantity of milk. In all three cases the oxytocic power of the blood was raised (Fig.2) but to different degrees. This may have been due to varying rates of absorption and/or varying rates of destruction or excretion. The oxytocic substance produced was labile in the same way as that found occurring naturally in cows near term.

The details of the three experiments are given below.

1. 5.9.33. Milkpail. Blood taken from the external jugular vein at 8.15 a.m. At 8.45 a.m. 10 c.c. of Pituitrin (Parke, Davis & Co.) were injected intramuscularly. Second blood sample taken at 12 noon.

The first blood sample contained much less than 1 o.u. per litre; 1 c.c. of the test solution gave no reaction whereas 0.05 c.c. of the standard solution gave a large contraction. The second blood sample contained about 4 o.u. per litre.

This cow weighed 1092 lb. or approximately 500 kg. and must have possessed nearly 40 litres of blood; the total oxytocic content on this basis would be about 160 units. Actually 100 o.u. were injected. This also suggested that the oxytocic substance was not posterior lobe hormone.

2. 14.9.33. Exactly the same experiment was repeated on Milk-pail. The first sample gave less than 0.7 o.u. per litre; the second obtained 2½ hours after injection of Pituitrin yielded about 2 o.u.per litre.

3. 17.9.33. Hopeful. The assay is given in sufficient detail in Fig.2. In this case the oxytocic value rose to about 2 per litre.

When pituitrin is injected intramuscularly there is an increase in the labile oxytocic substance in the blood. The increase is of short duration suggesting that this substance is either destroyed or excreted very quickly. Dale (1909) produced evidence that injected pituitrin is excreted in the urine.

Summary

With a few exceptions the oxytocic power of cows' blood is low; during the last week of pregnancy the oxytocic power increases. Immediately after parturition the oxytocic power again falls to a low value. The oxytocic substance is more labile than pituitrin from the posterior lobe of the pituitary but it is neither histamine nor calcium; its amount is increased for a short time by intramuscular injection of pituitrin.

Acknowledgments

Dr.N.C.Wright and Dr.R.C.Garry gave much valuable advice. Mr.A.B.Fowler assisted in the taking of samples and supplied the obstetrical histories. The cost of this work was borne mainly by the Hannah Dairy Research Institute and to a small extent by the D.C.Andrew Fund of the Institute of Physiology, Glasgow University.

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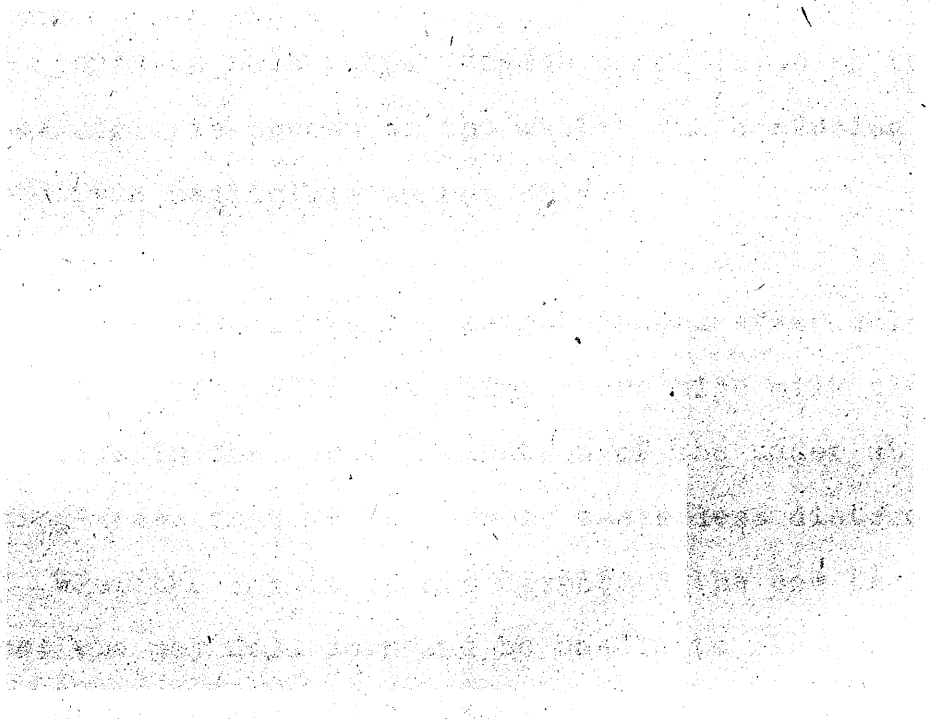

Chapter Three

THE OXYTOCIC PROPERTY OF THE BLOOD AND ITS RELATION TO MILK FEVER IN COWS

From the Hannah Dairy Research Institute, Kirkhill, Ayr, and the Institute of Physiology, University of Glasgow - 1934.

Milk fever is perhaps more than any other condition a disease of theories and it was therefore only human to test out another speculation during the course of the work described in the previous chapter. About four hundred papers have already appeared on this rather dramatic condition so that even if no new light is thrown on the matter the confusion will be increased by a negligible amount only.

Milk fever (paresis puerperalis) occurs in well nourished cows usually ten to seventy hours after calving. Almost invariably the affected animals are high milk yielders - the best cows in the byre. Palpation of the udder at the time of calving shows that it is in most cases less distended than normal. When the condition has developed the cow trembles and staggers; she may fall down and be unable to rise; the leg muscles become very rigid and no attention is paid to needle jags; coma and death supervene if the case is not treated.

It seems a fairly safe assumption that no such disease as milk fever existed two or three centuries ago. Its

appearance is associated with advance in methods of breeding applied to domestical animals - it might be termed a disease of civilisation. It seems to have been recognised first by Price (1806) and Skellet (1807) in this country according to Fish (1927). Before 1896 the theories as to the cause of milk fever were speculations of historical interest only. In that year Schmidt injected a one per cent solution of potassium iodide into the udder on the assumption that in the colostrum there was formed a toxin which gave rise to the paretic phenomena; his method was an immediate success. But the idea that there was an autointoxication had to be abandoned when it was found that injection of water or even of air was just as effective. In 1896, of course, the science of bacteriology was beginning to provide clues to all sorts of diseases and it was natural at that time to think in terms of infection. The name 'milk fever' is , however, a misnomer since there is usually no fever; more than this the condition is relieved within a few hours after blowing up the udder with air - this dramatic kind of recovery is never seen in an inflammatory process. The distension with air is now a standard procedure; the air from a bicycle pump is passed through some antiseptic solution and then through a blunt ended needle through the teat into the udder. Each of the four teats is tied with a tape to prevent escape of air; the tapes are removed in a few hours.

The next advances in our knowledge of this condition were delayed till the methods of blood chemistry had been developed. It was suggested about 1923 by a Canadian veterinarian (whose name is not recorded) soon after insulin was discovered that milk fever resembled closely the symptoms produced in man by excessive doses of insulin. Widmark & Carlens (1925) arrived at the same idea because they found that after blowing up the udder in normal cows the blood sugar was raised; they were able also to produce symptoms like those of milk fever by injection of several hundred units of insulin. This work was widely quoted by reviewers because the report was published in a chemical periodical and not in a relatively obscure veterinary journal. It has since been shown that the total blood sugar is normal or above normal (Fish, 1927 and Hayden, 1927); a method invented the year before enabled the glucose and the lactose to be estimated separately. Curiously enough it was found that injections of glucose occasionally relieved the condition. Hayden (1929) using improved methods showed that there is actually an increase in the glucose content of the blood in milk fever - on the average 63 mg. per 100 c.c. of blood compared with 41 mg. in normal blood. It will be seen that compared with normal human blood the sugar content is very low and the idea of a hypoglycaemia would be arrived at at once if no controls were taken. The normal standard of blood sugar in the

cow, goat, and sheep is definitely lower than that of man or dog. It is always dangerous to assume that man and animals resemble one another more than superficially.

Blendinger (1917) came to the conclusion that milk fever was a calcium inanition and he thought that estimations of calcium in milk and blood and brain should be made. In 1925 Dryerre & Greig wrote a paper reviewing the problem of milk fever and they hazarded a guess that it might be due to calcium deficiency; they compared the clinical findings of milk fever with those of incomplete parathyroprivea. (It seems rather unlikely that calcium deficiency has much to do with the condition; Fitch, Boyd, Eckles, Gullickson, Palmer, and Kennedy (1932) reported on a number of cows kept for three years on a low calcium diet - no mention was made of milk fever and there was no alteration in the level of calcium in the plasma as compared with animals kept on diets much richer in calcium). Curiously enough when Dryerre and Greig's paper appeared Little and Wright had been analysing samples of blood from cases of milk fever and they had found that the serum calcium was low; their paper was published a few months later in 1925. In 1926 these authors reported that they had given calcium salts by intravenous and intramuscular injection with success in cases of milk fever. Dryerre & Greig (1928 a and b) repeated and confirmed this work; they showed that injection of air raised the blood calcium about

10 p.c. above normal. This work was confirmed by Sjollema (1928) who found no other significant alterations in blood chemistry. It appears then that milk fever is a tetany produced by excessive loss of calcium from the mammary tissue; it should, however, be pointed out that it may occur before lactation is fully established. It has been calculated, however, that the secretion of half a gallon of colostrum would be sufficient to deplete the blood of the whole of its calcium if there were no reserve store from which the blood might be replenished. Many of the writers on milk fever do not accept entirely the parathyroid or tetany theory. In human tetany one of the characteristic signs is Chvostek's; when the facial nerve is lightly tapped as it crosses the jaw bone a spasm of the whole of that side of the face occurs. I tried this test out in cows in the acute stages of milk fever, but I was never able to convince myself that the resulting muscular twitch was greater than that normally found in healthy animals. Fish showed in 1929 that the inorganic phosphate of the blood was lowered in milk fever; after parathyroid extirpation it is slightly increased so that milk fever can hardly be considered due to parathyroid deficiency.

The real cause of milk fever has not yet been found. The most likely assumption is that it is an acute hormonal upset; otherwise it is difficult to understand why an injection

of calcium or blowing up the udder should be sufficient to tide the animal over the illness with only occasional recurrences. One might hazard a guess that some delay in recovering from the great endocrine disturbance of parturition brings on the condition. To put this matter to the test was the reason for extending the investigation described in Chapter Two of the subject of milk fever.

One of the difficulties about milk fever is that cases are very sporadic and occur without warning. It is not at all easy to obtain material. Farmers are naturally anxious to have the udders inflated at the earliest moment because their animals are valuable and milk fever cows, being high milk yielders, are especially valuable. The owners are also rather loath to let people experiment upon their animals; they were - in the present instance - apt to regard a small loss of blood from one of their beasts with more anxiety than I, in my experimental zeal, could understand. It is a great tribute to the tact and professional skill of Mr.W.A.Macgregor and Mr.David Weir, the two veterinary surgeons who were called in to the cases occurring in the farms that so many samples were obtained.

Methods

The method was exactly as described in the previous chapter. It was pointed out there that the oxytocic substance

was labile but that it seemed to remain in citrated blood which was kept in the ice chest. Several thermos flasks were kept ready each with a large boiling tube in the centre surrounded by ice; one was always taken home at night so as to be available for late calls. To avoid any loss the work was always begun immediately on returning from the farm.

Results

Controls. All the cases of milk fever occurred in the first week of the puerperium. The five animals mentioned in Chapter Two which showed no abnormal symptoms in the puerperium can be regarded as controls. None of the samples showed any measurable oxytocic value, i.e. the values were in every instance less than 1.0 o.u. (oxytocic units) per litre which was about the threshold of the method. A typical result is illustrated in Fig.1.

Milk fever cases. Case 1. Seventeen hours post partum. Sixth calf. Cow staggering but not comatose. Blood taken before inflation of the udder, contained 18 o.u. per litre (Fig.2).
Case 2. Three days post partum. The cow was lying on her side unable to rise but not comatose. Symptoms suggestive of milk fever had been noted twelve hours previously. Mammary blood taken before inflation contained 2 o.u. per litre.
Case 3. Ten hours post partum. The cow was stiff and staggering but not lying down. Jugular blood taken before inflation

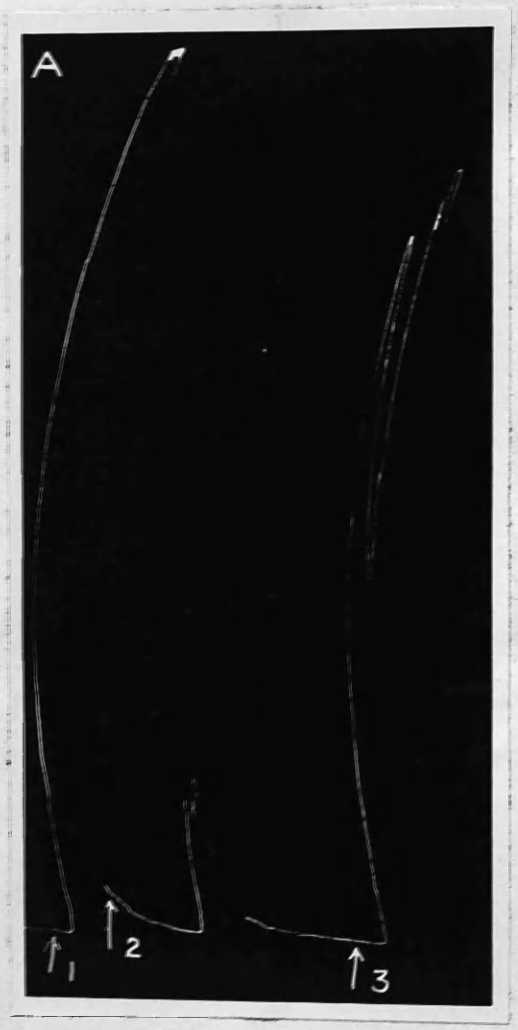

Fig. 1. T denotes the blood extract made in the manner indicated on page 86. S denotes the dilution of "pituitrin" containing 20 oxytocic units per litre. Control case, 24 hours after calving. 1. 0·2 c.c. S. 2. 2·5 c.c. T. 3. 0·1 c.c. S.

FIG. 2.

Fig. 2. T denotes the blood extract. S denotes the dilution of pituitrin containing 20 o.u. per litre. Milk fever case. 1. 1·0 c.c. S. 2. 1·3 c.c. T. 3. 1·0 c.c. S. 4. 1·1 c.c. T.

contained 3.3 o.u. per litre.

Case 4. Sixteen hours post partum. The cow was lying on her side, very stiff, but not comatose. She stood up after inflation of the udder. Jugular blood taken one hour after inflation contained 3.0 o.u. per litre.

Case 5. Udder inflated eighteen hours post partum. Recurrence of milk fever symptoms three days later. Cow was staggering about, muscles were very stiff but no coma. Jugular blood taken before inflation contained 2 o.u. per litre.

Case 6. Nothing abnormal was noted during the pregnancy (the fifth). After the calving it was suspected that there was some eversion of the uterus. Two hours later she lay on her side rigid, but not comatose. The udder was inflated after taking a blood sample. She was sick and did not eat well for several days.

Three days antepartum	Less than 1.0	o.u. per litre
Two hours " "	2.7	" " "
Two hours postpartum	3.5	" " "
Four hours " "	5.0	" " "
Seventeen hours post partum	2.5	" " "
Sixty five hours " "	Less than 1.0	" " "

Briefly these results show that the oxytocic power of the blood in milk fever is about the same as that which characterises blood obtained in the last week of pregnancy. There is not sufficient information to relate the oxytocic power to the severity of the symptoms.

Discussion

There is then an increase in the oxytocic content of the blood in cases of milk fever; a similar increase can be brought about by injection of pituitrin (Chapter Two). It is of interest to compare the biochemical results following on injection of pituitrin with the biochemical findings in milk fever summarised at the beginning of this chapter.

It has long been known that injection of posterior pituitary substance raises the blood sugar. It has been shown by Nitzescu & Benetato (1930) that pitressin produces in rabbits an increase in inorganic phosphate as well as a hyperglycaemia. Pitocin produces a slight rise of blood sugar without any alteration of phosphate. Urechia, Groze & Retezeanu (1930) state that there is a lowering of the blood calcium in man an hour after the intravenous injection of pitressin, with a small increase or decrease of inorganic phosphate. According to these authors pitocin has very little effect on blood calcium, but tends slightly to increase inorganic phosphate.

It is a common practice to starve for twenty four hours before delivery an animal which is likely to develop milk fever. Dryerre & Greig (1925) were of the opinion that a high protein diet favoured the occurrence of milk fever. Starvation has been shown by Maclean (1928) to reduce the oxytocic content

of the blood. Unfortunately from the point of view of the present theory there is no information as to the success or otherwise of this prophylaxis.

None of these considerations is inconsistent with the notion that a pituitary hormone plays a part in the causation of milk fever, that excess of this hormone in the blood at a time of hormonal adjustment and imbalance produces the changes which are responsible for the signs and symptoms of milk fever.

If this notion is correct it could be put to the test by injecting pituitrin into a recently calved animal. The high cost of experiments which involve the use of cattle excluded a lengthy investigation of this problem; smaller animals cannot be substituted for cattle since milk fever is a disease of very rare occurrence in animals other than the cow. There is, however, one rather inconclusive case to report.

This cow had had milk fever at three previous calvings. As she showed no signs of the condition 29 hours post partum a blood sample was taken and immediately thereafter 20 c.c. of pituitrin were injected intramuscularly. The blood sample was found to contain 15 o.u. per litre. Two hours after the injection of pituitrin another blood sample was taken; this contained less than 0.6 o.u. per litre. Not only did the injected pituitrin not raise the oxytocic value as was found in the work described in the previous chapter but it seemed to lower the

oxytocic power. On the next morning (i.e. two days post partum) a further blood sample was taken and immediately afterwards 10 c.c. of pituitrin were injected intramuscularly. The blood sample showed 10 o.u. per litre. A sample taken $2\frac{5}{4}$ hours later showed 4 o.u. per litre. Thus there was again a fall in the oxytocic power following injection of pituitrin. This cow did not at any time show signs of milk fever; she was sold and passed out of our observation after the second day.

The above results can be interpreted in two ways, but it is not possible to decide which is the likelier explanation. First, pituitrin injections will not produce milk fever and excess pituitary excretion is not the primary cause of this condition; second, an inappropriate dose was administered at an inappropriate moment. The latter possibility could be tried out except for the objections indicated above only if more animals were available but it has also to be remembered that the effect of pituitrin on metabolism varies with the dosage. Morris (1933) found that whereas small doses of pituitrin injected subcutaneously into goats caused an antidiuresis and a decrease in nitrogen excretion, large doses gave a diuresis and a large excretion of urinary nitrogen. It seems just possible then that the failure to induce milk fever may have been due to wrong choice of dose.

Summary

Six cases of milk fever in cows are reported in which the oxytocic content of the blood was found to average 3 o.u. per litre. Five controls showed no measurable oxytocic content. The biochemical findings in milk fever are compared with those occurring after injection of pituitrin. These suggest that milk fever may be due to excessive or continued secretion of pituitary hormone after parturition. An unsuccessful attempt to confirm this experimentally is described.

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Chapter Four

OXYTOCIC PROPERTIES OF BLOOD EXTRACTS AND THEIR PHYSIOLOGICAL SIGNIFICANCE.

From the Institute of Animal Genetics,
University of Edinburgh - 1935.

Towards the end of 1933 I read a paper on the work of the last two chapters at a joint meeting in Edinburgh of the West of Scotland Biochemical Club and the corresponding Edinburgh Society. After the meeting J.M.Robson, who was then working in Professor Crew's Institute of Animal Genetics, suggested that I should go further into the question. He was at that time working on the problem of parturition from another angle and he hoped that the oxytocic work would fill a gap in the picture which he had left untouched. Professor Cathcart allowed me to borrow the apparatus used the previous year at Ayr so that the work could be repeated and extended. Work began early in July, 1934, although a certain amount of preparatory work was done earlier. I was responsible for obtaining the blood samples from the cows, for the chemical preparation of the blood and for the guinea pig titrations while Robson carried out the mouse tests.

In Chapters Two and Three experiments were described which suggested that the blood removed from the cow towards the

end of pregnancy or during parturition contained a substance capable of causing contraction of the isolated guinea pig's uterus. As data relating to the oxytocic content of body fluids are of great interest in a consideration of the mechanisms concerned in the control of the uterine activity during gestation and at parturition it appeared desirable to obtain further information regarding the occurrence and nature of the active substance.

A series of experiments was, therefore, performed repeating exactly the technique described in Chapter Two and the following additional experiments were carried out as a help in the elucidation of the problem.

1. The blood of other species (the rabbit and the woman) was examined for its content of oxytocic substance and

2. The titration of all samples was performed not only on the uterus of the guinea pig, but also on the uterus of the mouse. In the latter case uterine strips were removed usually from animals either during or shortly following parturition; though in several experiments when parturient mice were not available uteri from oestrous mice were used. The sensitivity to oxytocin of the uterus of the parturient mouse is high (Robson, 1934) while that of the oestrous uterus, though not so great, is also well marked. The uterus of the mouse has the advantage of being unaffected by comparatively large amounts of

certain pharmacologically active substances (e.g. histamine, ergotoxine and choline derivatives), thus making it particularly suitable for the qualitative investigation of oxytocic substances.

Technique

Blood samples from cows and from women in labour were in all cases obtained by venesection. Mr.A.D.Buchanan Smith allowed me to take blood from the cows in the Shorthorn Dairy Herd at the University Farm; Dr.J.B.Dewar of the Simpson Memorial Hospital procured the human specimens. In the case of the rabbits samples of heart blood were obtained under ether anaesthesia; in one case indicated below blood from an ear vein was used. The preparation of the blood extracts was carried out exactly as described in Chapter Two - that is, the plasma proteins were precipitated by hydrochloric acid with subsequent boiling.

Virgin dioestrous guinea pig uteri were suspended in Burn-Dale solution through which a mixture of oxygen with 5 p.c. CO_2 was bubbled. The mouse uterine strips were suspended in Ringer-Locke solution through which oxygen was bubbled. The temperature and the rate of bubbling were maintained constant. The capacity of the containers was 100 c.c. Preparations of the posterior pituitary lobe "Pituitrin" and "Pitocin" (kindly supplied by Dr.White of Parke, Davis and Co.) were used.

Results

Twenty one samples were removed from pregnant or parturient cows and one sample from a non-pregnant cow, and the content of oxytocic substance of the extract determined on the guinea pig and mouse uterus. The results are given in Table I. An examination of these data shows:-

1. That the content of oxytocic substance of the blood from pregnant and parturient cows, as determined on the guinea pig uterus, is in the great majority of cases low. For, out of twelve samples taken in the last twelve days of pregnancy only three showed detectable amounts of oxytocic substance which assayed respectively at values corresponding to 4, 4, and 1 oxytocic units per litre of the original blood.

2. That the assay performed on the mouse uterus gave a higher proportion of positive results and higher values for the content of oxytocic substance. Thus the samples which assayed at 4, 4, and 1 units per litre on the guinea pig uterus gave values of 20, 8, and 10 units per litre respectively when tested on the mouse uterus. Moreover, several samples in which no detectable amounts of oxytocic substance were present according to the guinea pig test gave values up to 20 units per litre when assayed on the uterus of the mouse.

3. That three samples obtained, two from animals in the earlier stages of gestation and one from a non-pregnant cow gave

TABLE I.

Name of Cow	Non-pregnant	Days before parturition: 54	47	12	11	10	8	7	6	5	4	3	2	1	0	+1
1. Loanhead											20;-					<3;-
2. Cowslip II.									<3;-				<5;-		<5;-	
3. Melody						10;-		10;1								
4. Gay Lustre									<5;<1.5							
5. Bounty										<1.5; <1						
6. Captivation				20;4	<2;<2			<3;<1	<6;<1.5			2;<2				
7. Dosie										5;<3					20;<3	
8. Captive	<2; <2															
9. Rosie Belle							1;<2		8;4			<10;<3				
10. Heroine			<6; <2													
11. Westcraig		<6;<5														

Oxytocic content of cow blood samples (expressed as oxytocic units per litre) measured on the mouse and guinea pig uterus in vitro. The value obtained on the mouse uterus is given first in all cases. 0 means within 24 hours preceding parturition; +1 means the day following parturition.

negative results both on the mouse and guinea pig.

Six samples were obtained from women in the second stage of labour with strong pains. The results of assay of oxytocic substance on the guinea and mouse are given in Table II. It will be seen that the guinea pig tests gave negative results in all but one sample which assayed at 5 oxytocic units per litre. On the mouse uterus three of the samples yielded positive results, giving values of 20, 10, and 2 oxytocic units per litre. It will again be noted that in the one case giving a positive value in the guinea pig, a much higher value was obtained when the test was performed on the mouse.

TABLE II.

Oxytocic content of human blood samples taken during the second stage of labour (expressed as oxytocic units per litre) measured on the mouse and guinea pig uterus.

Case	1	2	3	4	5	6
Guinea pig	5	<2	<7	<2	<2	<2
Mouse	20	<6	2	<5	10	<5

Samples were obtained from eight rabbits and gave the following results.

1. Three non-pregnant animals (R01, R05, C25) showed no

detectable amounts of oxytocic substance on the guinea pig (<3, <2.5, <5 o.u. respectively). The extract from C25 tested on the mouse gave a value equivalent to more than 10 o.u. per litre (the sample from C25 was the only blood sample taken from the vein, all the other rabbit samples were taken from the heart). Another non-pregnant rabbit (C19) gave less than 6 o.u. per litre when tested on the mouse.

2. Four animals were injected with posterior lobe extracts namely:-

R02 received 0.5 c.c. of Pituitrin intramuscularly and one hour later the blood was taken and assayed at 3 o.u.per litre when tested on the guinea pig uterus.

R03 treated in exactly the same way assayed 8 o.u.per litre on the guinea pig uterus.

R06 received 1 c.c. of Pitocin intramuscularly and one hour later the blood assayed 40 o.u. per litre on the mouse and 10 o.u. per litre on the guinea pig.

C25 was injected intramuscularly with 1 c.c. of Pitocin immediately after the taking of the sample already referred to, and one hour later a further sample showed no detectable amounts of oxytocic substance (<5 o.u. per litre) when tested both on the mouse and guinea pig uterus.

3. Rabbit RA463 was hypophysectomised on the 25th.day of pregnancy and aborted 33 hours later. The blood was taken immediately after abortion and assayed more than 20 o.u. on the mouse and less than 10 o.u. on the guinea pig.

In experiments described in Chapter Two I found that the oxytocic substance present in the blood during the last

stages of pregnancy and parturition was unstable and that an active extract soon lost its potency when kept in the ice chest. A similar fate did not overtake oxytocin when added to blood in vitro. It was, therefore, thought advisable to determine by tests on the uterus of the mouse as well as of the guinea pig whether the extracts obtained in the present experiments had been similarly affected by storage. The results of the assays are given in Table III.

TABLE III. showing effect of storing in ice chest on content of oxytocic substance as tested on the mouse and the guinea pig uterus. Values expressed as oxytocic units per litre.

Animal	First day		Second day		Third day		Fourth day	
	Mouse	G.pig	Mouse	G.pig	Mouse	G.pig	Mouse	G.pig
Rabbit R02	-	3.0	-	-	-	-	-	<2.4
Rabbit R03	-	8.0	-	-	-	<2.4	-	-
Rabbit R06	40	10	>20	8	>20	10	>20	<5
Rabbit Ra463	>20	-	7.5	-	15	-	-	-
Cow 6	20	5	10	<4	-	-	-	-
Woman 1	20	5	20	10	>20	5	-	-

It will be seen that no extract decreased in oxytocic when the test was performed on the uterus of the mouse; but when tested on that of the guinea pig there is definite evidence that the amount of oxytocic substance that can be demonstrated in a given

extract decreases on standing.

A number of causes might account for such results but, on the supposition that the same active substance was responsible for causing the contraction of both the mouse and guinea pig uterus, it seemed likely that the disappearance of activity towards the uterus of the guinea pig might be due to the development in the extract of a factor which inhibited the response of the guinea pig uterus to oxytocic substances, but exerted no such effect (or a much less effect) on the response of the mouse uterus. Experimental investigation of this hypothesis provided very adequate evidence for the existence of an inhibitory substance of this nature.

It was found that an extract, even immediately after its preparation, may in some experiments inhibit to a certain extent the reaction of the guinea pig uterus to a given dose of oxytocin. This is illustrated in Fig.1, which shows the inhibition of the response to a dose of oxytocin by 1 c.c. of a freshly prepared extract added to the bath just previously. Such an extract, however, exerts no inhibitory action on the response of the uterus of the mouse to oxytocin.

When an extract is allowed to stand in the ice chest the amount of inhibitory substance in it appears to increase so that the addition of 1 c.c. of it to the bath may completely abolish the response of the guinea pig uterus to a supraminimal

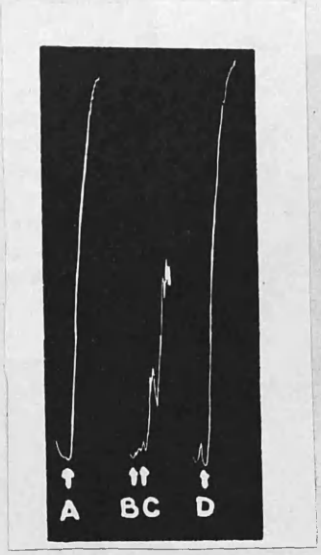

Fig. 1. Showing inhibition of the response of the guinea-pig uterus to oxytocin by a freshly prepared extract of cow blood. A, 0·006 unit of pituitrin added to bath; B, 1·0 c.c. of cow 8 blood extract added to bath; C, 0·006 unit of pituitrin added to bath; D, 0·006 unit of pituitrin added to bath. Solution changed after each addition of pituitrin.

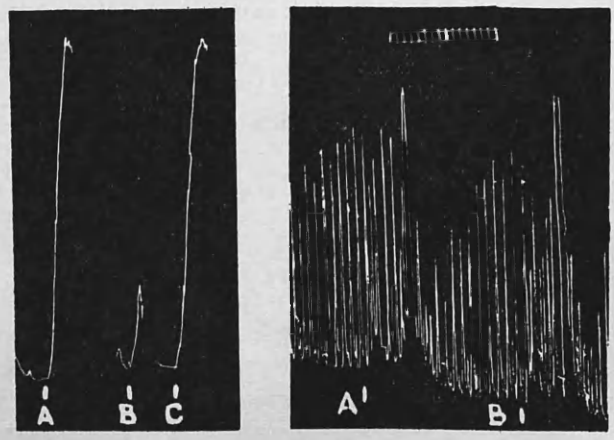

Fig. 2. Showing inhibition of the reaction of the guinea-pig uterus to oxytocin by small dose of stored blood extract and absence of inhibition in the mouse with a larger dose of the same extract. First tracing, guinea-pig uterus: A, 0·007 unit of pituitrin added to bath; B, 0·5 c.c. of cow 10 blood extract (stored for five days in ice chest) plus 0·01 unit of pitocin added to bath; C, 0·007 unit of pituitrin added to bath. Second tracing. uterus of parturient mouse: A, 0·02 unit of pituitrin added to bath; B, 1·0 c.c. of cow blood extract (as above) plus 0·02 unit of pituitrin added to bath. Solution changed after each addition of pituitrin. Time interval = 1 min. for both tracings.

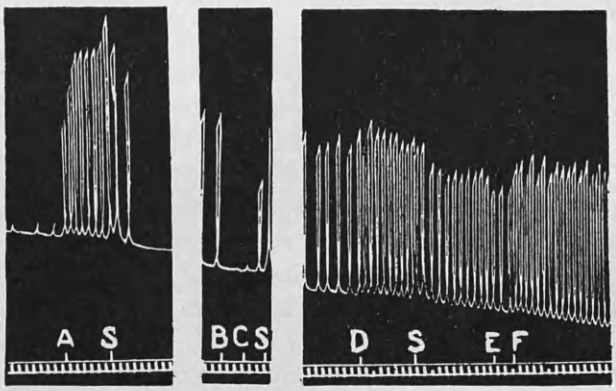

Fig. 3. Showing inhibition of the reaction of the parturient mouse uterus to oxytocin by large doses of stored blood extract. A, 0·01 unit pituitrin added to bath; B, 2 c.c. of cow 8 blood extract (stored for three days) added to bath; C, 0·01 unit pituitrin added to bath; D, 0·01 unit pituitrin added to bath; E, 1 c.c. cow blood extract as above added to bath; F, 0·01 unit of pituitrin added to bath. *S*, solution changed. Time interval = 1 min.

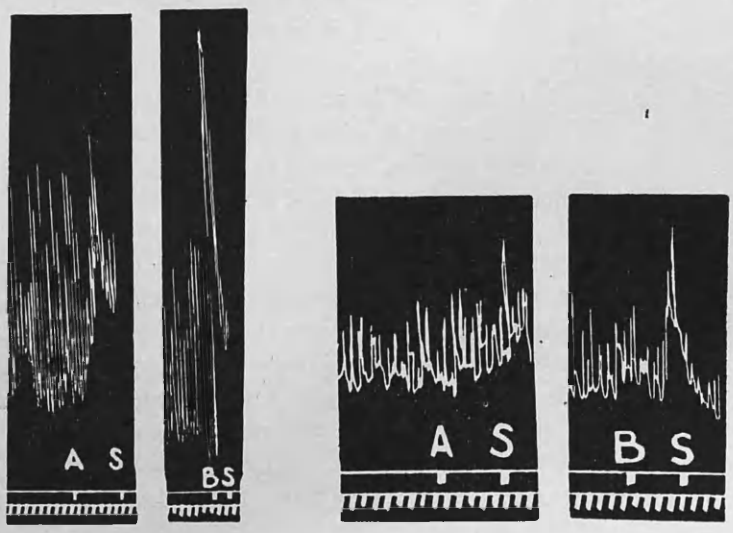

Fig. 4 *a*. Fig. 4 *b*.

Fig. 4. Illustrating the difference in the character of the response of the mouse uterus to oxytocin and to an active blood extract. (*a*) A, 0·01 unit pituitrin added to bath; B, 2 c.c. cow 6 blood extract added to bath. (*b*) A, 0·02 unit of pituitrin added to bath; B, 1 c.c. cow 7 blood extract added to bath. *S*, solution changed. Time interval = 1 min.

dose of oxytocin and even 0.5 c.c. may have a marked inhibitory effect. This is illustrated in Fig.2. Moreover, such an extract may exert some inhibitory effect on the response of the mouse uterus too, although here the degree of inhibition is less marked. This is illustrated in Fig.3.

DISCUSSION

The results of these experiments show definitely that an oxytocic substance capable of causing contractions of the isolated uteri of the guinea pig and of the mouse can occasionally be demonstrated in extracts of blood when the investigation is performed under the given conditions, and two main questions then arise, namely:-

1. Whether the appearance of this substance bears any time relation to the state of the animal, with special reference to gestation and parturition;

and

2. What the nature of the oxytocic substance is, and especially whether it is identical with the oxytocic substance of the posterior lobe.

An examination of the data does not support the view that the occurrence of the oxytocic substance is a factor related to the initiation of parturition. It will be noted that although the results bear a superficial resemblance to those obtained at Ayr a year before fewer positive results have been

obtained in the present series of assays. In Chapter Two, however, no claim to have found the oxytocic substance responsible for parturition was made. It is impossible to say how far the change from the Ayrshire to the Shorthorn cows affected the results. Considering first the evidence offered by the action of cow blood extracts on the guinea pig uterus, it will be seen that the sample obtained actually during parturition gave no positive effect; and further the only two cows that gave comparatively high values, namely 4 o.u. per litre given by Cow 6 on the 12th.day ante partum and Cow 9 (6 days ante partum) gave negative findings at subsequent stages of pregnancy. In the case of Cow 6 indeed these negative results were obtained no less than four times on the 11th., 7th., 6th. and 3rd.days ante partum. Positive effects were obtained more frequently when the extracts were tested on the mouse uterus but, nevertheless, the data do not support the view that these positive effects occur only with samples withdrawn at specific times. It is to be noted that three samples withdrawn from two cows not in the latest stages of gestation and from one non-pregnant cow gave negative results both on the mouse and guinea pig uteri, but these data are not sufficient to warrant the conclusion that the oxytocic substance when present occurs only during the latest stages of pregnancy.

The evidence obtained from the examination of the human

material supports on the whole the results obtained from the cow for only one out of six specimens obtained from patients definitely in the second stage of labour gave any effect on the guinea pig uterus while three out of six gave an action on the uterus of the mouse.

The results obtained on the rabbit show that no oxytocic substance that is active when tested on the guinea pig uterus is present in non-pregnant animals but that such samples may occasionally yield a positive effect when tested on the mouse uterus (animal C25).

The present series of experiments fully bears out the finding (already described in Chapter Two) that active extracts when kept in the ice chest for some time fail to elicit contractions of the guinea pig uterus but in view of the additional evidence brought forward a new interpretation of these results appears necessary.

The facts to be considered are:-

1. That the oxytocic effect on the mouse uterus is not lost on standing, and
2. That extracts which have stood for some time in the ice chest contain a factor which inhibits the response of the guinea pig uterus to oxytocin (and in larger quantities that of the mouse uterus to oxytocin).

The most likely conclusion appears to be that the observed loss of potency on standing is not due to the destruction nor inactivation of the oxytocic substance but to the effect

of the inhibitory factor which has developed.

Furthermore a careful examination of the experimental details offers an explanation for the previous finding that Pituitrin added to blood does not become inactivated on standing. For in the earlier experiments the quantity of Pituitrin added (0.02 units per c.c.) was such that the actual assay involved the addition to the bath of considerably less than 1 c.c. of extract - the guinea pig uterus in vitro will often respond to 0.002 units. Now in the present experiment it has been found that a definite inhibitory effect on the guinea pig uterus can only be demonstrated with about 0.5 to 1 c.c. of extract while 2 c.c. or more are necessary for the mouse uterus. Hence under the experimental conditions described in Chapter Two no inhibitory action would come into play, and the activity on standing would, therefore, remain unchanged. On the other hand in the case of the oxytocic substance in the blood the quantities are smaller, the amount of extract added to the bath in order to elicit a contraction larger and the effect of the inhibitory substance becomes evident.

In so far as the nature of the oxytocic substance is concerned a number of possibilities can be eliminated. The properties of the mouse preparation exclude histamine; the substance is not a choline derivative as its effect persists after atropinisation. A consideration of the quantities involved makes

it highly unlikely that calcium or potassium is responsible. Control experiments with Locke solution treated in exactly the same way as the plasma have eliminated osmotic phenomena. In view of the marked differences in the titration values on the guinea pig and mouse uterus it can be concluded that if one substance is responsible for both effects it cannot be identical with the oxytocin of the posterior pituitary lobe. Such a conclusion is supported by an examination of the contraction curve of the mouse uterus under the influence of the oxytocic material of the blood extracts; this curve (Fig.4) rises more steeply and falls more rapidly than that produced by oxytocin (i.e. Pituitrin).

Clark found in 1924 that certain decomposition products of proteose cause contraction of the uteri of both the rat and the guinea pig and that the titration values using the rat uterus were considerably larger than those obtained with the guinea pig uterus. This is of great interest, more especially as the active extracts used by Clark could be obtained by means of acid treatment. It appears possible that the substance dealt with in the present investigation is of a similar nature, its production being dependent on the acid treatment of the plasma. Olivecrona (1920) found that a demonstrable stimulation could be produced by 0.00013 p.c. of peptone. If we make various assumptions in a rough calculation it will be seen that this value is just possible in the present work. If it is supposed that 99.9 p.c.

of the protein is precipitated leaving 0.1 p.c. as peptone, then, since each c.c. of plasma contains 0.08 g. protein and gives 1.4 c.c. acid extract, each c.c. of acid extract would yield about 0.00006 g. of peptone. 2 c.c. of this extract added to the bath of 100 c.c. would give the concentration quoted by Olivecrona.

Even if protein decomposition products are responsible for the uterine contractions this offers no explanation of the fact that some bloods give active extracts and that others do not, or of the difference in the frequency of positive results in Chapters Two and Four. I feel that, although the description of the results of the second experiment may seem to deny to a large extent the findings of Chapter Two, yet examination of the actual results will show that the present results differ in degree rather than in kind. For example, non-pregnant animals in both series gave negative findings whereas some at least of the animals in late pregnancy gave positive results; further after injection of pituitrin the oxytocic content of rabbit's blood was increased just as was the cow's blood in the previous experiment. There is undoubtedly some factor missing but unfortunately the experiments give no inkling of its nature.

Whatever the nature of the oxytocic substance the possibility that the extract does contain small amounts of oxytocin cannot be excluded. Indeed it is of interest to consider

what quantities of oxytocin could be expected in a blood extract, arguing from 'a priori' considerations. The well known clinical observation that intramuscular injection of 5 or less units of oxytocin cause contraction of the parturient uterus finds confirmation in the experiments of Bourne & Burn (1928) and of Moir (1934) on the human uterus near term. Since the blood volume of a woman is on the average about 5 litres the actual concentration in the blood could never be greater than 1 unit per litre or 0.1 unit per 100 c.c. and is in all likelihood very much smaller. Schübel & Gehlen (1933) working on the early puerperal uterus of the cat in vivo showed that motor effects could be elicited with concentrations of oxytocin of less than 0.01 unit per litre of the circulating blood; they also found that the threshold dose for eliciting uterine contractions by intramuscular injection is about ten times the intravenous threshold dose. It is quite likely then that - presuming for the moment that posterior lobe hormone is the oxytocic agent responsible for parturition - the amount of oxytocin in the blood at parturition is less than 0.1 unit per litre. A similar conclusion follows from a consideration of experiments (Robson, 1933a and b, 1934) with uterine strips in vitro which in the case of man, rabbit and mouse show minimum reactions to doses of oxytocin of 0.01 to 0.1 unit per litre when the strips are removed from parturient subjects. All these considerations

support the conclusion that if an oxytocic substance is involved in the process of parturition its concentration in the blood might be not more than a few tenths of a unit per litre and is highly unlikely to be more than one unit per litre. The demonstrations of such amounts would involve either the use of a reactor even more sensitive than the guinea pig uterus, or some method of concentrating the active substance without incidentally producing oxytocic agents not previously present as such in the blood, and at the same time the method would have to eliminate to any serious extent the action of inhibitory agents.

SUMMARY

1. The oxytocic activity of extracts prepared from the blood of (a) pregnant cows, (b) women in the second stage of labour, and (c) rabbits before and after injection of posterior pituitary extracts, was tested simultaneously on the isolated uterus of the guinea pig and of the mouse.

2. A number of these were found to possess oxytocic properties; this property does not appear to be associated with any particular phase of gestation. The uterus of the mouse was consistently found to be more sensitive to the active substance than the uterus of the guinea pig.

3. A factor capable of inhibiting the 'in vitro' reaction to oxytocin of the uterus (more especially that of the guinea pig) has been demonstrated in blood extracts. The inhibitory power of these extracts appears to increase on standing.

4. The nature of the oxytocic substance and the significance of its occurrence are discussed.

The expense of this investigation has been defrayed by grants from the Medical Research Council and the Carnegie Trust. Professor A.J.Clark, F.R.S., kindly discussed the results and made some valuable suggestions.

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Chapter Five

THE REACTION OF THE GUINEA PIG UTERUS IN VITRO AFTER INTENSIVE TREATMENT WITH OESTRONE

from the Department of Physiology, University of Bristol, 1935.

As indicated in Chapter Four the only hope of a solution to the problem of the oxytocin content of blood lies in finding a uterus which is remarkably sensitive to the hormones of the posterior lobe and remarkably insensitive to all other agents, such as histamine, which possess, one is tempted to say accidentally, the property of causing the uterus to contract.

The experiments to be described were carried out in the early part of 1935. At that time it seemed that the main function of oestrin (apart from oestrus itself) was to render the uterus sensitive to oxytocin so that parturition could be brought about in due season. This idea arose from examination of the rabbit uterus, very little work having been done on other uteri. It was, therefore, quite reasonable to think that by excessive dosage of oestrin the uterus might be made very sensitive to oxytocin, and to hope that it might at the same time become less sensitive to other oxytocic agents. It must be emphasised that the following experiments are pharmacological in nature and were not intended as an attack on the problem of

parturition; nevertheless it will be seen that the results fall into line with those obtained later.

Method

The guinea pig uterus in vitro is probably the most sensitive reactor to oxytocin. It was thought best to start with a sensitive uterus and to attempt to make it more sensitive. It would be interesting to know why Burn and Dale (1922) chose it for their method of standardising pituitary extracts. It has a great disadvantage in its sensitivity to histamine - a disadvantage not possessed by the mouse uterus (this has already been discussed in Chap.Four). To make the experiments as uniform as possible mature animals about 400 g. in weight were used. A short time after spaying the left uterine horn was removed under general anaesthesia; it was placed at once in the bath and its reactions to oxytocin and histamine tested. Each animal then received an intensive course of oestrone injections; at the end of this period it was killed and the remaining uterine horn tested out in vitro.

Burn-Dale solution was used in 60 c.c. containers in a thermostatically controlled bath the design of which will be described later as it had not yet reached its final form. The oxytocin was kindly supplied by Dr.White of Parke, Davis & Co. The histamine used was ergamine acid phosphate which is put up

in convenient tablets by Burroughs Wellcome. The oestrone (British Drug Houses) was dissolved in hot absolute alcohol so that each c.c. contained 1 mg. When diluted with 9 parts of water a 10 p.c. solution or rather fine suspension of oestrone was obtained. This was injected subcutaneously at two hour intervals. This is rather a heroic procedure both for the animal and for the experimenter, especially when, as in one case, it was carried out over 48 hours without a break. In the last two experiments this two hourly treatment was preceded by a prolonged course of oestrone in oil. The alcoholic solution (1 mg.per c.c.) was added to the appropriate amount of sterilised olive oil and then the alcohol was blown off in a stream of air directed across the wide mouth of the bottle which was heated gently at the same time. The oil became quite clear when all the alcohol disappeared. This is the standard procedure for making solutions of oestrone in oil and it was used throughout this work. It will be seen in later chapters that sesame oil has taken the place of olive oil. I have never been able to understand why the change has become popular because both oils are quite bland. The vehicle is of great importance since it governs to a large extent the rate of absorption of the oestrone; if results which can be compared with other published work are required then it is better to use the more fashionable sesame. This fashion had not yet declared itself at the time of the present experiments. (A somewhat

parallel occurrence is the use of clove oil by Barcroft in his manometer. It is probable, knowing Sir Joseph, that clove oil happened to be handy on a nearby shelf. It is vile stuff but so strong is the clove oil tradition that few have had the courage in public to recommend liquid paraffin).

Results

Guinea pig A. 420 g.

11/2/35. In oestrus. Spayed.
13/2/35. Left horn in vitro; reacted to 0.02 o.u. (oxytocic units) and 0.01 mg. histamine.
16/2/35. Injected with 0.1 c.c. of 1:10 solution of 1 mg. oestrone per c.c. of absolute alcohol at 10, 12, 2, 4, 6, 8.30 (i.e. 60 γ). Killed 9.30 p.m. Reacted to 0.01 o.u. pitocin; 0.01 mg. histamine, no effect.

Guinea pig C. c. 400 g.

18/2/35. Not in oestrus. Spayed.
20/2/35. Left horn of uterus in vitro reacted to 0.0075 o.u. Pitocin; 0.0025 mg. histamine.
24/2/35. Injected with 0.1 c.c. of 1:10 solution of oestrone at 9, 11, 1, 3, 5, 7, 7, 9, 11, (then on 25/2/35) 1, 3, 5, 7, 9. Total dose of oestrone 130 γ over 24 hours. Killed and put in vitro at 11.15 a.m. The uterus was large and juicy. It reacted to 0.02 o.u. Pitocin and to 0.005 mg. histamine.

Guinea pig D. 470 g.

18/2/35. Spayed.
25/2/35. Left horn in vitro. Reacted to 0.03 o.u. and to 0.005 mg. histamine. After several doses of histamine reacted to 0.01 o.u.
28/2/35. 0.2 c.c. of 1:10 oestrone, i.e. 20 γ, at 9, 11, 1, 3, 5, 7, 9, 11, 3, 5, 7, 9, 11, 1, 3, 4.30 p.m. (0.32 mg. oestrone altogether). Killed 7 p.m. Right horn in vitro reacted to 0.005 o.u. pitocin and 0.002 mg. histamine.

Guinea pig E. About 400 g.

25/2/35. Spayed.
11/3/35. Left horn in vitro. Reacted to 0.01 o.u. and 0.0025 mg. histamine.
13/3/35. Injected with 30 γ oestrone (1:10 solution) at 10, 12, 2, 4, 6, 8, 10, 12, 2, 4, 6, 8, 10, 12, 2, 4, 6, 8, 10, 12, 2, 4, 6, 8, 10, 12 (i.e. 26 x 0.03 = 0.78 mg. oestrone). The vagina was still closed after all these injections. Killed at 1.30 p.m. on
15/3/35. Uterus reacted to 0.005 o.u. pitocin and 0.002 mg. histamine.

Guinea pig H. About 400 g.

18/3/35. Spayed.
20/3/35. Hemihysterectomy. Reacted to 0.005 o.u. and 0.001 histamine.
27/3/35. Injected twice daily with oestrone in olive oil - each injection 0.2 c.c. containing 0.05 mg. oestrone. Repeated daily till
3/4/35. Total dose in oil now 0.7 mg.
4/4/35. 0.2 c.c. of 1:10 oestrone solution from 10 a.m. two hourly till 8 a.m. on
6/4/35. Total dose in 1:10 soln. = 24 x 0.02 = 0.48 mg. Grand total 1.18 mg. oestrone. Vagina open. Killed at 10 a.m. Uterus very large and congested. Mouth of vagina much congested. The smear contained cornified cells, leucocytes, and red blood cells. The uterus in vitro reacted to 0.004 o.u. pitocin and then to 0.001 histamine, then to 0.001 o.u.

Guinea pig G. About 400 g.

18/3/35. Spayed.
20/3/35. Hemihysterectomy. Reacted to 0.0025 o.u. of pitocin and 0.001 mg. histamine.
27/3/35 to 10/4/35. Two injections of 0.2 c.c. oil per day, each 0.2 c.c. containing 0.05 mg. oestrone. Total dose = 1.4 mg.
11/4/35. 0.2 c.c. of 1:10 oestrone solution from 2.30 p.m. two hourly till
13/4/35. at 8 a.m. Total dose of 1:10 solution = 22 x 0.02 = 0.44 mg. oestrone. Grand total 1.84 mg. oestrone. Killed at 10 a.m. Left horn filled with clear fluid, cyst like, no blood. Reacted to 0.001 o.u. and 0.001 histamine.

TABLE I. Threshold doses in vitro.

	Pitocin		Histamine	
	Before	After	Before	After
A	0.02	0.01	0.01	0.01
C	0.0075	0.02	0.0025	0.005
D	0.03	0.005	0.005	0.002
E	0.01	0.005	0.0025	0.002
H	0.005	0.001	0.001	0.001
G	0.0025	0.001	0.001	0.001

The summary of results in Table I. indicates that while the oestrone treatment made the uterus a little more sensitive to oxytocin without increasing its sensitivity to histamine the lowering of the threshold was not sufficient to justify a renewal of the work on the oxytocic content of the blood especially as the labour involved is all out of proportion to the gain in discrimination. In 1941 one could probably achieve the same result by one injection of oestradiol dipropionate but even this simplification would not justify a new attack. For several reasons a much greater increase in sensitivity is necessary. Many of the blood samples in Chapters Two and Four were reported negative which means that their oxytocic content was below the threshold of the method; further, to get rid of the inhibitory effects of large amounts of serum and osmotic upsets the sensitivity must be much greater. I think that a minimum of a 25 times increase in sensitivity would justify re-opening the question of the oxytocic property of the

blood especially if the method were of general application so that tests could be carried out on uteri of several species at the same time.

On the then current theories of parturition the highest sensitivity to oxytocin would be expected at parturition but here again it was found that the sensitivity was of the same order as before with no discrimination in favour of oxytocin. We shall return to this question in a later chapter.

Guinea pig G2. 20/5/35. Two hours post partum. Only one foetus. Anterior half of pregnant horn in vitro reacted to 0.003 o.u. and 0.003 mg. histamine. Non-pregnant horn reacted to 0.002 o.u. and 0.002 histamine.

This absence of discrimination between oxytocin and other oxytocic materials is one of the theoretical objections to the oxytocin theory of the causation of parturition. It is thought by some learned critics that the uterus might achieve something more subtle than a mere general increase in sensitivity.

Summary and Conclusions

An attempt has been made to make the uterus of the guinea pig more sensitive to the posterior pituitary extract by very intensive treatment with oestrone. Although this was so excessive as to produce frank bleeding in one case the increase in sensitivity varied from 1/3 to 6 times with usually little increase in sensitivity to histamine. There is nothing to be gained by re-opening the question of the oxytocic property with

this small increase.

(The matter still lies in this impasse in 1941, and the matter is still one of urgency).

This work was carried out with the aid of a grant from the Medical Research Council.

Reference to Chapter Five

Burn, J.H. and Dale, H.H. (1922).
Sp.Rep.Ser.Med.Res.Council No.69.

Chapter Six

THE EFFECT OF CERTAIN HORMONES ON THE ACTIVITY OF THE UTERINE MUSCLE OF THE GUINEA PIG

(This work was carried out first in the Department of Physiology, University of Bristol, then in the Department of Pharmacology, University of Edinburgh, and completed in the Institute of Physiology, University of Glasgow, during 1935 and 1936).

Since the frontal attack on the problem of parturition seemed to offer no hope of advance attention was next directed to the control of uterine movements during pregnancy and parturition. At the time of this work the rabbit was the only animal which had been thoroughly investigated. In view of the differences between the reproductive phenomena of the rabbit and those of other species it was quite possible that the reproductive hormones would have different effects in other species. It was also considered important to make records in the living animal instead of examining the activity of surviving strips of uterine muscle in vitro.

Removal of the corpus luteum activity during pregnancy is followed by different results according to the species investigated. In some species, such as the mouse and the rabbit, gestation is interrupted, whilst in others such as the guinea pig, the mare, the cat and the woman, pregnancy may continue to full term. Hence the luteal secretion is necessary for the maintenance of pregnancy in the first series of animals, whilst in

the second series the corpus luteum is not an essential organ; though possibly the luteal hormone is secreted elsewhere. Indeed the finding of small amounts of this hormone by Adler, de Fremery & Tausk (1934) in the placenta of woman and cow suggests that this organ may be another site of production; also the results obtained by Courrier & Gros (1935) in the cat and by Selye, Collip & Thomson (1935) in the rat offer strong evidence that progestin may be elaborated in the uterine contents.

The exact nature of the action exerted by the luteal hormone in the maintenance of pregnancy has by no means been worked out, but it appears possible that two alterations in the physiological activity of the uterine muscle are of significance. These effects of progestin are (1) desensitisation of the muscle, i.e. inhibition of its reaction to the oxytocic hormone of the posterior pituitary lobe (Knaus, 1930; Robson & Illingworth, 1931; Robson, 1935), and (2) inhibition of the rhythmic contractions of the muscle exhibited in the living animal after injection of oestrin (Reynolds & Allen, 1932). The first of these effects has so far been definitely observed only in the rabbit in which the alterations in the reactivity can be seen both in vitro and in vivo. In the other rodents which have been examined up to this time the luteal hormone exerts no desensitising action on the uterine muscle in vitro (Siegmund, 1930) but the reactivity of the muscle in the intact animal has not yet been

studied. Inhibition of the rhythmic contractions of the uterus by progestin has been investigated only in the rabbit, in which this effect is obtained in vivo but not on the isolated muscle (Reynolds & Allen, 1932; Robson, 1935). The action of the hormone on the intact uterus of other animals has not yet been determined, though indirect and rather contradictory evidence has been obtained in the human subject from investigation of uterine activity at various stages of the menstrual cycle (Knaus, 1935; Moir, 1934).

The object of the present investigation was to find out whether the luteal hormone exerts any action on the uterine activity of an animal other than the rabbit. Information was sought on four points, namely, (1) whether the reactivity to oxytocin measured in vivo is affected; (2) whether any effects can be demonstrated on the reactivity in vitro when this is measured quantitatively; (3) whether the rhythmic contractions recorded in vivo are inhibited; and (4) whether there is any effect on the spontaneous contractions in vitro.

In addition a gonadotropic preparation was tested to see whether the gonadotropic hormones might directly affect uterine function, as it has been suggested (Reynolds, 1932) that they may exert a controlling influence on the uterine activity in animals in which removal of the ovaries during pregnancy does

not lead to abortion.

Methods and Materials

The experiments were performed on thirty three virgin guinea pigs about 250 to 300 g. in weight. All the animals were ovariectomized by the dorsal route.

Injection of oestrin was always commenced on the day following operation, except in the two uninjected controls. Crystalline oestrone in solution in oil was used.

The following preparations of corpus luteum hormone were given: (1) crude extract (Robson & Illingworth, 1931) made from bovine corpora lutea from the Glasgow abattoir and referred to in Table I. as "own"; 1 kg. of corpora yielded some 60 c.c. of this extract; about 7 c.c. of the extract gave full progestational proliferation and inhibition of the uterine reactivity and motility in the mature rabbit; (2) purified preparations in solution in oil received from Organon Laboratories, standardised on the immature rabbit; (3) crystalline progesterone (I am indebted for this and the purified preparations to Dr.Tausk of Organon Laboratories); (4) Proluton kindly supplied by Messrs. Schering (1 rabbit unit of Proluton = 0.75 mg. progesterone dissolved in oil).

A gonadotropic preparation, referred to as M2 in Table I., made from pregnancy urine was used, the rabbit

ovulating dose of which was approximately 0.5 mg. This was administered in solution in saline.

All these injections were made subcutaneously.

The experiments were made on four groups of ovariectomised guinea pigs: (1) animals receiving no injections; (2) animals receiving oestrone only; (3) animals injected with oestrone and progestin; and (4) animals injected with oestrone and gonadotropic hormone.

All animals (except group 1) received the course of oestrone spread over three or seven days, 0.01 mg. of the hormone in 0.1 c.c. of oil being given each day, morning and evening. The last injection of oestrone was in all cases made three days before the examination of the uterus. In the second group the animals thus received no injections during the two days preceding the experiment. In the third group progestin was given twice daily for the three days immediately preceding the experiment, the total amount being indicated in Tables I. and II. In the fourth group gonadotropic hormone was administered twice daily on the three days before the experiment; in addition GP 20 and GP 21 received a dose on the day of experiment; the total amounts are also given in Tables I. and II.

Following the period of injections the animals were operated on under ether anaesthesia, except GP 25, 26, 27, 28 and 31, in which the experiment was performed under chloralose

anaesthesia. In the majority of experiments one uterine horn was removed and cut in halves which were used for duplicate determinations in vitro. The other horn was used for the experiment in vivo. Two methods were used for recording the uterine activity in vivo. In the first the variations in the intrauterine volume were registered, while in the second the contractions of the longitudinal muscle were recorded. The second method was elaborated with the object of obtaining a very high degree of sensitivity as it was to be used in ovariectomised untreated animals.

In the first method an incision was made in the lower part of the abdominal wall, the bladder was emptied and separated from the vagina. A small glass cannula was introduced through the vagina and cervix and tied in position in the lower part of the uterine horn as indicated in the lower left part of Fig.1. A small incision was then made in the upper part of the horn and warm Locke solution was perfused by way of the side tube through the cannula and allowed to escape at the incision until all the air was displaced. A ligature was then tied around the uterus immediately below the incision; the side tube was clamped and the abdomen was then closed. In two animals GP 11, and GP 13, the uterine horns were larger than usual and the caudal half only was used for the determinations in vivo in order to keep the height of the expected contractions within the limits of the recording

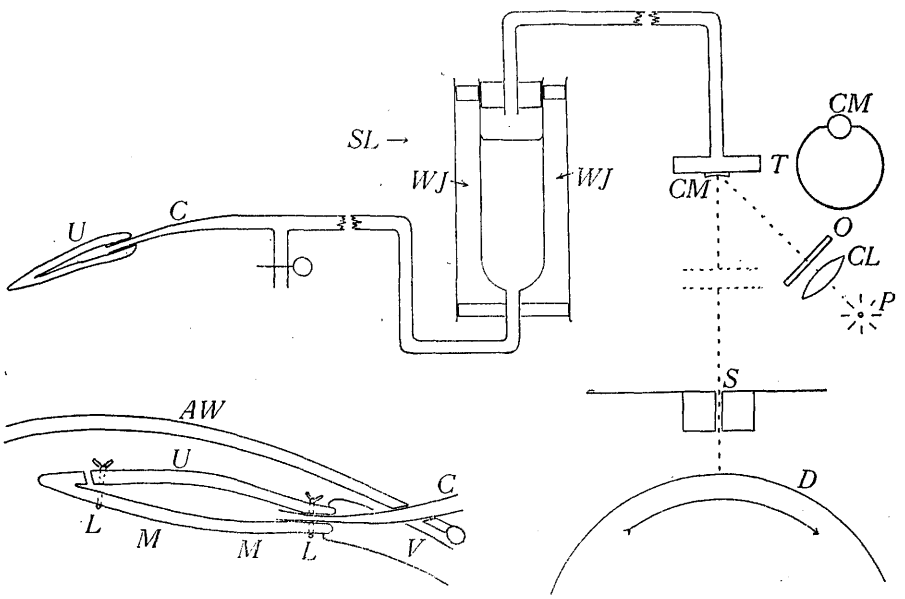

Fig. 1. Diagram of apparatus used to record movements of circular muscle *in vivo*. *U*, uterus; *C*, cannula; *M*, mesentery; *L*, ligature; *V*, vagina; *SL*, saline level; *WJ*, water jacket; *CM*, concave mirror; *T*, tambour; *P*, pointolite; *CL*, condensing lens; *O*, slit; *S*, camera slit; *D*, drum carrying bromide paper; *AW*, abdominal wall.

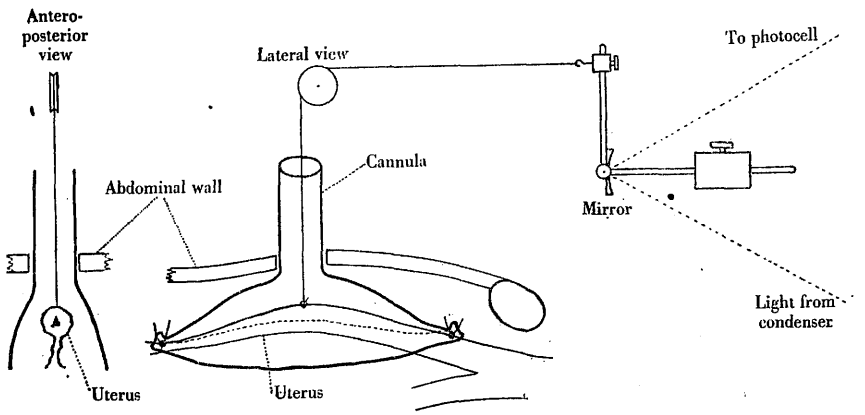

Fig. 2. Diagram of cannula used to fix uterus in the registration of the movements of the longitudinal muscle *in vivo*.

camera. Throughout the operative interference great care was taken not to disturb the blood supply of the uterus. The uterine cannula was connected to a reservoir containing Locke solution as in Fig.1, there being a continuous fluid connection. The height of the fluid in the reservoir was adjusted to give about 10 cm. of pressure of water in the uterus. The air space in the upper part of the reservoir was connected by rubber tubing to a tambour of 3.8 cm. diameter covered with a thin rubber diaphragm. A mirror was fixed on a flattened edge of the circumference of the diaphragm and optical records were made on bromide paper on a slowly moving drum. The electrically driven drum was enclosed in a wooden box with a slit in one face formed by two square brass rods; the walls of the slit were painted dull black to reduce reflection and to decrease the area from which stray light could enter the camera. In spite of this precaution it was necessary to build a kind of tent of brown paper over the lamp, mirror and camera. In this way quite black lines were obtained on perfectly clean background. Injection of 0.1 c.c. of fluid into the cannula produced a movement of the horizontal line of light on the vertical camera slit of 2.5 cm. The distance between CM and S in Fig.1 was 1 metre.

It is worth while to make some remarks about the optical side of the recording system as it does not seem to be mentioned in the text books and I have often found that even physicists

were unacquainted with the practical details. An image of the pointolite P is thrown on CM by the condensing lens CL. One way of getting a good image of the slit O on S is to use a surface silvered concave mirror or a stainless steel mirror; the former deteriorates very quickly while the latter is more expensive and the light reflected from it is of poor actinic value. The best solution (incidentally used, but kept as a commercial secret, in various electrocardiographs) is to obtain a glass lens with equal and opposite curvatures on the two sides (concave on one side and equally convex on the other). When the convex surface is silvered and varnished we have a back silvered concave mirror which will remain bright almost indefinitely. The mirror is mounted on a three pointed piece of brass foil (shim) fastened to the tambour with Chatterton or other fixative; when the points are turned over the mirror is held firmly. When the apparatus is set up it will be found that there are three images of the slit on the camera, one bright and two dim; one of the dim images comes from the front surface of the mirror, the bright one comes from the back surface of the mirror, the origin of the second dim image is possibly reflection from the anterior surface posteriorly on to the back silvered surface. If the mirror is now rotated in its shim star a position will be found in which the three images are in one straight line - this is the position used to make the photographs.

In the second method the upper and lower ends of one

uterine horn were tied to the extremities of a specially devised boat shaped glass cannula covering the organ and thus preventing its being affected by the movements of the other abdominal viscera (see Fig.2). (It is of interest to note that this cannula was adopted after a trial of the Cushny myograph had shown that it was not suitable; the glass boat shaped cannula on the other hand has been of very great value in many uterine investigations ranging from the mouse to the monkey. It has failed miserably in attempts to record intestinal movements; it is difficult to see the reason for this failure because the anatomical construction of the uterine horns of lower animals and of their intestine is not essentially different. It may be that the later modifications of the cannula would be more satisfactory but this has not yet been tried). A thread attached to the mid point of the uterus was passed through the centre of the cannula (the funnel) and carried over a pulley to an isotonic lever with a mirror at its fulcrum. The abdomen was then closed over the cannula.

The beam of light reflected from the mirror fell on the cathode of a photocell (see Fig.3). The image of the object (window A) was of the same shape and size as the cathode of the photocell and rotation of the mirror led to an increase or decrease in the area of the photosensitive surface illuminated. The photoelectric current passed through a high resistance GL in series with a dry battery of 60 volts. The voltage drop in GL

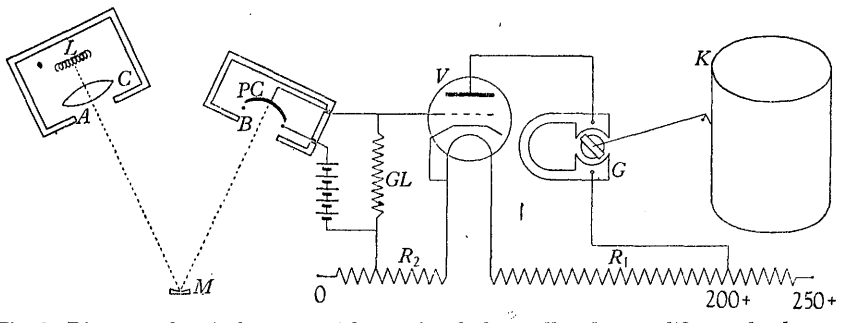

Fig. 3. Diagram of optical system with associated photocell, valve amplifier, and galvanometer writing on smoked drum. *L*, talkie exciter lamp (10 v., 7·5 amp., G.E.C.); *C*, large lantern condenser; *M*, mirror fixed to isotonic lever; *PC*, photocell CMG 8 (Osram); *GL*, grid leak (0·25–1·8 megohms); *V*, DL valve (Osram); *G*, moving coil galvanometer; *K*, smoked drum; R_1 and R_2, voltage dropping resistances connected across mains in series with cathode heater of valve; *A* and *B*, windows in front of the condenser and in front of the photocell which are both of the same shape and size as the cathode of the photocell.

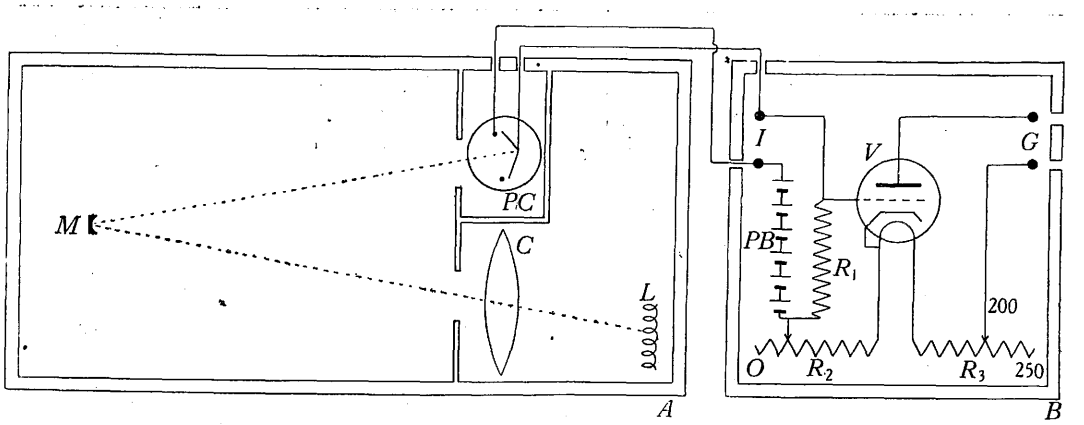

Fig. 2. Diagram (not to scale) of photocell apparatus and associated amplifier

was arranged to oppose the negative bias on the grid of valve V obtained from the tapping on R_2. The galvanometer G writing on a smoked drum was connected between the anode of the valve V and a tapping on R_1 at 200 volts. An increase in the area of illumination of the photocell results in an increase of anode current of V, and so the pointer of the galvanometer rises up on the drum, by an amount proportional to the increase in the illumination. The excursions recorded on the smoked paper are thus directly proportional to the movements of the uterus.

In actual practice it is best to separate the amplifier from the rest of the apparatus as shown in Fig.3a. The amplifier can then be used with any other piece of apparatus and indeed it may be termed the heart of our lecture demonstration apparatus (see Bell, Bell, Knox & Smellie, 1937). The optical apparatus is kept in a long box as indicated on the left of Fig.3a and illustrated in Fig.3b where a tambour is shown in the position of the isotonic lever of the present experiments.

At first a large sized moving coil galvanometer with a long counterpoised pointer was used and its movements were oil damped. The slow contractions of the uterus did not require an instrument of high natural frequency. The recording of small slow changes was assisted by vibrations produced by a buzzer attached to the galvanometer stand. When this apparatus was demonstrated to the Physiological Society Prof.Winton drew my

attention to the advantages of using a Weston moving coil relay provided with much stronger springs so that a full scale deflection is obtained with about 40 mA. instead of 0.5 mA (see Winton, 1936). This writes very well on smoked paper without the annoyance of the continual buzzing. It has in addition quite a high natural frequency so that if it is provided with a very light glass pointer it will make quite passable records of the human electrocardiogram (see Bell, Bell, Knox & Smellie, 1937). The galvanometer is illustrated in Fig.3c.

This system is capable of providing a high degree of magnification - 1000 times or more when a lever is used; its sensitivity may be conveniently altered by optical methods or by changing the value of GL. The method possesses, in addition, the very great advantage of providing an immediate visual record of the contractions of the muscle without the long delay between recording and developing inevitable in a system employing ordinary photographic registration. The cost of the apparatus is soon saved in the change from photographic paper to smoked paper. It is important to keep the light source constant and a variable resistance of about 1 ohm and an ammeter are included in the lamp circuit.

Before the recording began the external jugular vein was exposed preparatory to intravenous injection by means of a fine needle on a 1 c.c. syringe.

The excised strips were suspended in oxygenated Locke

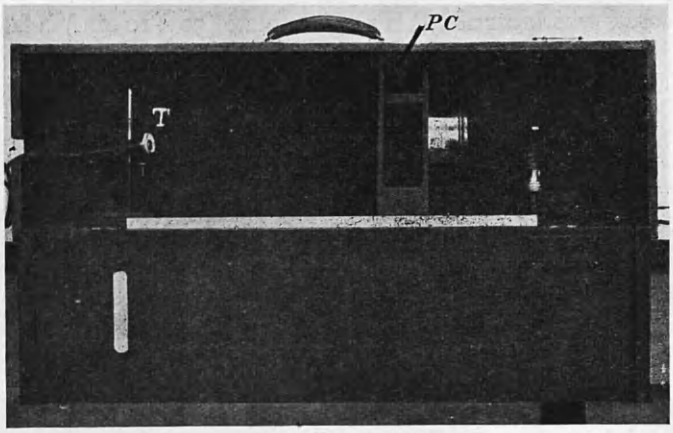

Fig. 3b. Photograph of box *A*. *T* is the tambour carrying mirror *M*. *PC* is the photocell

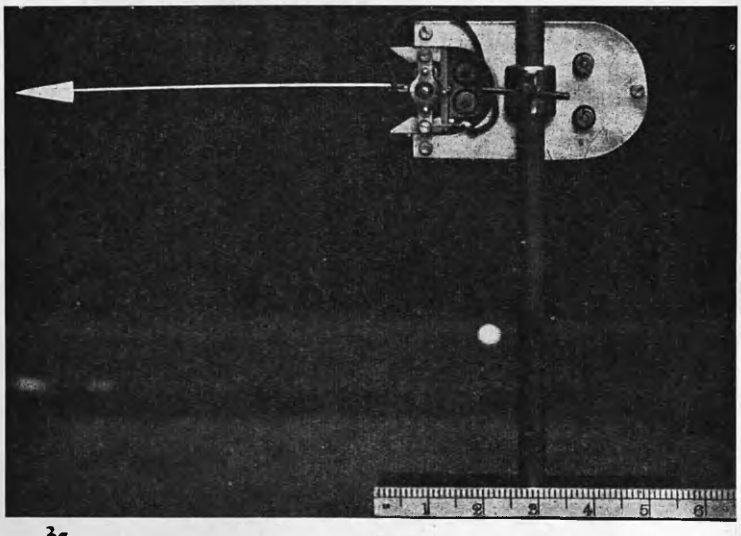

Fig. 3c. Weston moving-coil relay modified to write on smoked glass

solution at 37.5°C. in 100 c.c. containers.

A oxytocic preparation of the posterior pituitary lobe, pitocin (kindly supplied by Dr.White of Parke, Davis & Co.), was used to test the reactivity both in vivo and in vitro.

In all experiments an attempt was made to estimate the smallest amount of oxytocic hormone required to elicit a definite motor effect and this was taken as a measure of the reactivity of the muscle. The initial dose added in vitro was usually 0.005 unit. This was decreased until the minimum effective dose was found. In the experiments in vivo only a limited number of injections (usually not more than two) could be made. An attempt was made to give doses above and below the threshold amounts but this was achieved only in some of the experiments.

The spontaneous rhythmic activity both in vitro and in vivo was measured directly on the tracings in cm. and the data given in the text and Table I, represent these measurements. But in the experiments in which the photoelectric method of recording was used the actual downward movement of the centre of the uterus was calculated and is given in mm. in Table II.

Results

(a) In vivo:

The type of response obtained in vivo stands in marked contrast to the characteristic response of the guinea pig uterus to oxytocin in vitro. Usually the chief effect in vivo was an

increase in the frequency of the contraction waves, with no rise of the base line. When the rhythmic contractions were initially large then an effective dose of oxytocin caused no increase or even a decrease in the height of the individual waves. These effects are illustrated in Fig.4, in which the response of the uterus of animal GP 15 to the intravenous injection of 0.01 unit of oxytocin is shown, and also in Fig.5 which shows the response of the longitudinal muscle in vivo to the intravenous injection of 0.02 unit in animal GP 32. On the other hand if the waves were initially small the action of oxytocin included an increase in the height of the contractions. It was occasionally observed that the introduction of a needle into the vein was immediately followed by a single contraction of the uterus even when no injection was made. This is possibly of reflex nature and has to be distinguished from the true action of oxytocin.

An examination of the data obtained by the first method of recording (see Table I.) suggests that the reactivity exhibited in vivo shows no appreciable differences between the three groups investigated; there is no suggestion that administration of the luteal hormone has desensitised the uterus (see Figs.6 and 7).

Measurements of the spontaneous activity in vivo reveal no appreciable differences in amplitude or in frequency of the contraction waves between the various groups of animals (i.e. guinea

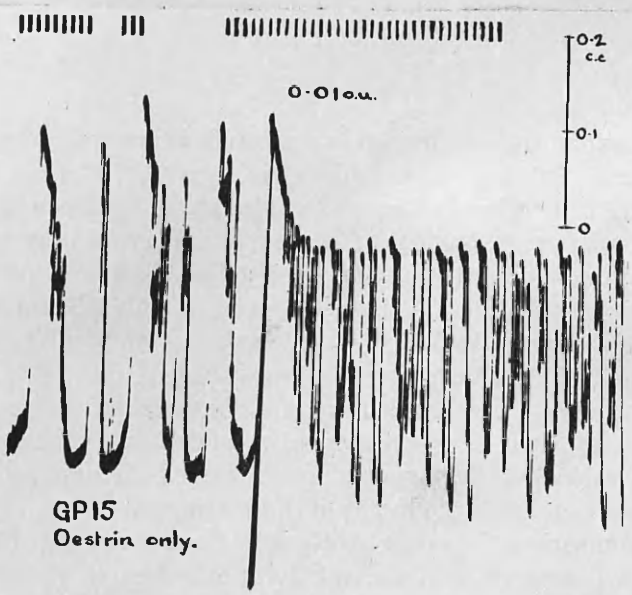

Fig. 4. GP 15. Animal treated with œstrin only. Response of the circular muscle of the uterus *in vivo* to the injection of 0·01 unit of oxytocin intravenously. Time intervals, 1 min.

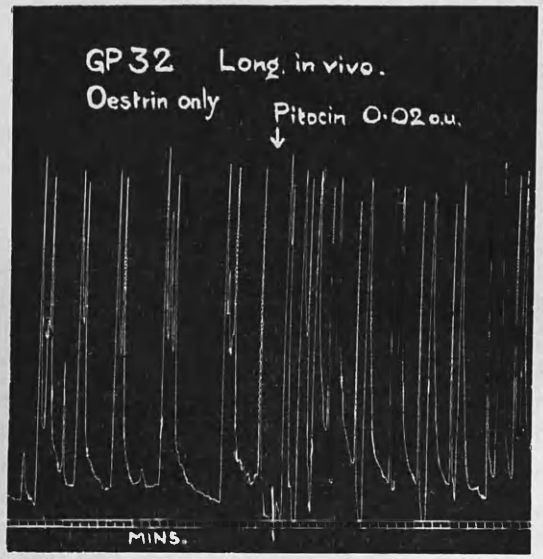

Fig. 5. GP 32. Animal treated with œstrin only. Response of the longitudinal muscle of the uterus *in vivo* to the intravenous injection of 0·02 unit of oxytocin. Time intervals, 1 min.

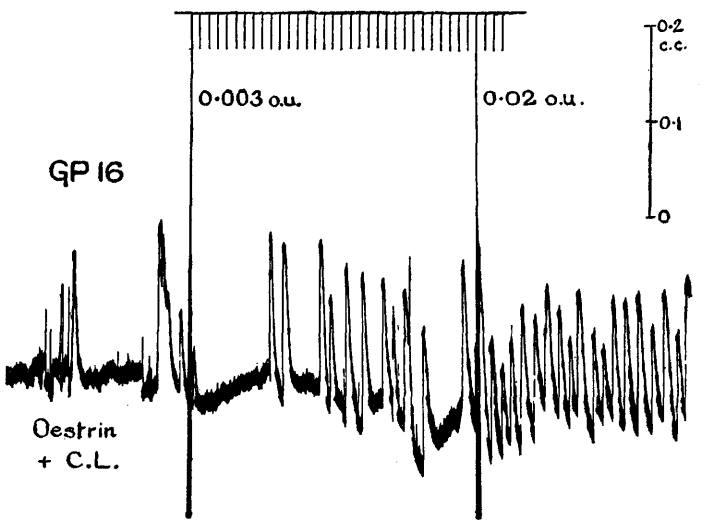

Fig. 6. GP 16. Animal treated with œstrin and corpus luteum extract. Response of the circular muscle of the uterus *in vivo* to 0·02 unit of oxytocin but not to 0·003 unit intravenously. Time intervals, 1 min.

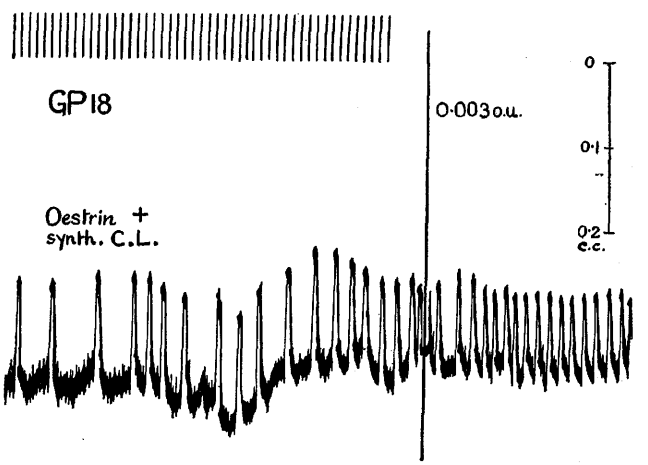

Fig. 7. GP 18. Animal treated with œstrin and progesterone. Absence of any response of circular muscle to intravenous injection of 0·003 unit of oxytocin. Notice the rhythmic contractions. Time intervals, 1 min.

pigs injected with oestrin only; those receiving oestrin and progestin; those receiving oestrin and gonadotropic hormone). If averages of the amplitudes for the various groups are taken then the figures obtained for the spontaneous activity in these groups are respectively 5.6, 5.0 and 9.0 cm.

In all but two of the experiments spontaneous contractions at approximately the maximum level began shortly after the introduction of the uterine cannula. In the two cases, however, in which only half the horn was used for determinations in vivo (G.P.11 and G.P.13) the rhythmic contractions did not attain their full height until about an hour after the beginning of the experiment; it is to be noted that the results for spontaneous activity actually recorded photographically in these two cases have been multiplied by two for the purpose of comparison with the other figures in Table I. The results obtained on the longitudinal muscle in vivo (Table II.) agree with those obtained on the circular muscle by the first method. It will be seen that quite marked rhythmic contractions have been obtained in one untreated ovariectomised animal (GP.35).

(b) <u>In vitro</u>:

Certain features of the records obtained in vitro are worthy of comment. In the first place the base line was invariably level; the muscle always relaxed to its original length after the changes of solution following contractions produced by oxytocin. Further the amplitude of the spontaneous rhythmic

TABLE I. Summarizing all findings except those n which the longitudinal muscle was examined *in vivo*

Animal no. GP	Oestrin injection: Days	Oestrin injection: Total amount mg.	Progestin: Days	Progestin: Total amount	Reactivity to oxytocin: *In vitro*	Reactivity to oxytocin: *In vivo*	Spontaneous activity: *In vitro*	Spontaneous activity: *In vivo*	Remarks: Size of uterus	Remarks: Smear
2	3	0·06	—	—	—	—	—	8·0	Fairly large	Dicestrous
6	3	0·06	—	—	—	0·004	—	5·0	Small	Dicestrous
7	3	0·06	—	—	<0·005	>0·004	3·5; —	2·0	Small	Pro-œstrous
9	3	0·06	—	—	0·002	>0·01	1·5; 2·0	5·0	Smaller than medium	Pro-œstrous
14	3	0·06	—	—	0·001	>0·01	0·1; 0·5	4·0	Medium small	Early pro-œstrous
3	8	0·16	—	—	—	>0·004	—	5·0	Medium	Dicestrous
4	7	0·14	—	—	—	—	—	3·5	Small	Not recorded
15	7	0·14	—	—	0·001	<0·01>0·003	0·4; 0·7	10·0	Large	Pro-œstrous
17	7	0·14	—	—	0·002	0·003	1·0; 1·8	6·0	Moderate	Early pro-œstrous
23	7	0·14	—	—	0·001	0·003	0·1; 0·2	7·5	Medium	Dicestrous
10	3	0·06	3	6 c.c. own	0·002	—	2·0; 2·5	7·0	Moderately large	Dicestrous
5	7	0·14	3	6 c.c. own	0·0005	—	1·5; 1·5	—	Large	Dicestrous
8	7	0·14	3	6 c.c. own	0·001	<0·02>0·003	0·8; 0·8	7·0	Moderately large	Dicestrous
11	7	0·14	3	6 R.U. Organon	0·0005	<0·003>0·001	1·0; 2·0	4×2=8	Large, only half used	Dicestrous
12	7	0·14	3	6 R.U. Organon	0·002	<0·01>0·003	nil	4·0	Large	Dicestrous
13	7	0·14	3	6 R.U. Organon	0·002	<0·003>0·001	0·5; 3·0	2×2=4	Large, half used	Dicestrous
16	7	0·14	3	6 R.U. Organon	0·002	<0·02>0·003	0·1; 0·3	4·0	Moderately large	Dicestrous
18	7	0·14	3	6 mg. progesterone	0·001	>0·01	0·2; 0·6	3·5	Medium	Dicestrous
22	7	0·14	3	6 mg. progesterone	0·002	>0·02	0·7; 1·5	2·5	Largish	Dicestrous
				Gonadotropic hormone						
20	7	0·14	4	16 mg. M2	0·002	>0·003	0·2; 0·4	9·0	Medium	Pro-œstrous
21	7	0·14	4	17 mg. M2	0·002	>0·01	0·7; 1·0	8·0	Moderate	Not recorded
19	7	0·14	3	30 mg. M2	0·0005	>0·003	0·2; 0·4	10·0	Medium	Dicestrous

contractions was very small in the majority of the experiments (see Table I.). Moreover a change of solution (after addition of oxytocin to the bath) was not followed by an increase, and often was followed by a decrease, in the height of the spontaneous contractions as compared with the period preceding the addition of the drug; a typical series of responses is illustrated in Fig.8.

Since it is well known that the uterus from the oestrous guinea pig exhibits very marked spontaneous activity in vitro and since a similar high activity has been shown to result from the administration of oestrin to the living animal (Marrian & Newton, 1933) these results needed further investigation. It appeared possible that the absence of any marked spontaneous activity might be due to the interval of three days elapsing between the last injection of oestrone and the time of the experiment. But this was found not to be the case. The fact that the uterine horns had always been cut into two parts in the present experiments seemed a likely explanation of the absence of large spontaneous contractions in vitro. A comparison was, therefore, made between the rhythmic activity of the whole horn and that of the half-horns removed from animals treated with oestrin over seven days, the experiment being performed three days after the last injection of oestrin. It was found that the whole horn gave, as was expected, very marked contractions. On the other

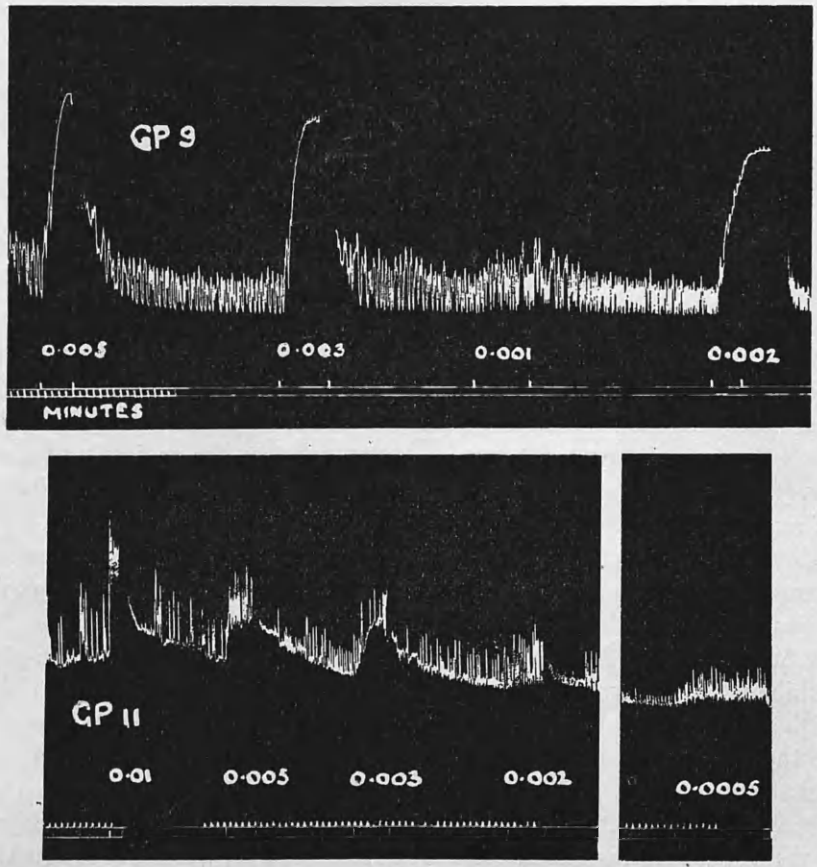

Fig. 8. Illustrating the reactivity to oxytocin and the spontaneous rhythmic activity of the uterine half-horns suspended in oxygenated Ringer-Locke solution. The solution was changed after every addition of drug. Time intervals, 1 min.

hand, the half horns from the same animals showed, as in the previous experiments, small contractions. It would thus seem that cutting the horn in half results in a very marked diminution of its rhythmic contractility in vitro. This relative quiescence of half horns is useful in pharmacological investigations of the uterus and students in my senior class are now advised to use half horns instead of whole horns in their experiments on uterine activity in vitro.

The doses of oxytocin needed to produce motor effects in vitro were comparatively small in all the experiments; thus no uterus failed to react to 0.005 unit per 100 c.c. and in the majority of experiments 0.002 unit was effective. In three cases a response was obtained with 0.0005 unit per 100 c.c. There was no appreciable difference between the spontaneous rhythmic activity exhibited in vitro by the uteri of groups 2, 3 and 4 (i.e. guinea pigs treated with oestrin, with oestrin plus progestin, and with oestrin plus gonadotropic hormone); and the reactivity to oxytocin in vitro was of the same order in these three groups.

A remarkable feature of the reaction to oxytocin is the marked difference between the concentrations of the drug necessary to cause contraction in vitro and in vivo. The concentrations in vivo (see Table III.) have been calculated on the assumption that the blood volume of the guinea pigs used was 20 c.c. The third column of Table III. has been obtained by dividing the

TABLE II Showing the results in animals in which the longitudinal muscle movements were recorded *in vivo*

Animal no. GP	Oestrin Days	Oestrin Total mg.	Progestin (Proluton) Days	Progestin (Proluton) Total	Reactivity to oxytocin *in vivo*	Spontaneous activity *in vivo* mm.	Remarks
34	—	—	—	—	0·01	0·5	Uterus small. Smear dioestrous
35	—	—	—	—	0·01	9·0	Uterus medium. Smear dioestrous
32	7	0·14	—	—	<0·02; >0·003	5·0	Uterus medium
33	7	0·14	—	—	0·02	5·0	—
36	7	0·14	3	6 R.U.	—	9·2	Uterus small. Smear dioestrous
37	7	0·14	3	6 R.U.	—	10·0	Uterus medium. Smear dioestrous

TABLE III. Comparison of reactivity *in vitro* and *in vivo*. The reactivity has been expressed as the effective dose of oxytocin per 100 c.c. of Ringer-Locke solution or per 100 c.c. blood.

GP	Reactivity *In vitro*	Reactivity *In vivo*	Ratio of concentration *in vitro*/*in vivo*
14	0·001	>0·05	>50
9	0·002	>0·05	>25
7	<0·005	>0·02	>4
15	0·001	<0·05>0·015	15 to 50
23	0·001	0·015	15 or less
17	0·002	0·015	7·5 or less
18	0·001	>0·05	>50
22	0·002	>0·1	>50
5	0·0005	<0·1>0·015	30 to 200
8	0·001	<0·1>0·015	15 to 100
16	0·002	<0·1>0·015	7·5 to 50
11	0·0005	<0·015>0·005	10 to 30
12	0·002	<0·05>0·015	7·5 to 25
13	0·002	<0·015>0·005	2·5 to 7·5
19	0·0005	>0·015	>30
21	0·002	>0·05	>25
20	0·002	>0·015	>7·5

effective concentrations of oxytocin in the blood (i.e. oxytocic units per 100 c.c. of blood) by the minimum effective dose in 100 c.c. Locke solution. It will be seen that in more than half the experiments the concentration necessary to cause contraction in vivo is more than fifteen times the effective concentration in vitro. In three cases the discrepancy is greater than fifty times.

Vaginal smears were taken from all the animals on the day of the experiment. In no case was a full oestrous smear obtained, although some of the animals showed various stages of pro-oestrous (see Table I.) All the smears from animals injected with progestin in addition to oestrin were, however, completely dioestrous, i.e., they contained epithelial cells and leucocytes.

DISCUSSION

Since this investigation was carried out essentially to determine the possible action of the corpus luteum in the maintenance of pregnancy in an animal other than the rabbit the results obtained with progestin will be examined first. There seems little room for doubt that, under the experimental conditions described, progestin has no appreciable inhibitory action either on the reactivity to oxytocin or on the spontaneous rhythmic activity of the uterine muscle in the guinea pig. This statement holds both for the investigation carried out in vitro

and for that performed in vivo. Before we can apply these results to the condition of the animal during pregnancy it is necessary to determine whether the doses of hormone used are within physiological limits. In so far as oestrin is concerned, it is obvious that the amounts used were not equal to those normally involved in the production of oestrus, since a full vaginal cornification was never induced. Hence, although we have no information as to the amount of this hormone produced during pregnancy, we can at least say that the quantities used in these experiments were well within the known physiological limits. There is unfortunately no information as to what constitutes a physiological dose either of oestrin or progestin in the guinea pig. (The case of oestrin will be dealt with in a later chapter). The amounts of progestin used would have been more than sufficient to produce the known actions of progestin in a rabbit weighing eight times more than some of the guinea pigs used. In the mature ovariectomised rabbit treated with oestrin the subsequent administration of 0.75 mg. of progesterone over four days results in a progestational proliferation of the endometrium similar to that seen at the height of pseudopregnancy; the same dose of progesterone also produces a desensitisation of the uterine muscle in vitro and in vivo, and inhibition of the spontaneous activity of the muscle observed in vivo. (Robson, 1936). Hence in the two animals injected with pure progesterone some eight

times the amount fully effective in the mature rabbit was given, and allowing for the difference in the body weight the dose in the guinea pig was actually more than fifty times that necessary to cause proliferation of the endometrium and desensitisation and inhibition of motility in the rabbit. Direct evidence that the doses of progestin used were, at least to some extent, effective in the guinea pig is shown by the fact that in none of the animals treated with this hormone in addition to oestrin were oestrous changes produced in the vaginal smear - this is an inhibitory action of progestin similar to that recorded by de Fremery, Kober & Tausk (1934) in the mouse.

These results, therefore, suggest that progestin does not, in the guinea pig, cause any inhibitory effect on the uterine activity similar to that described in the rabbit, and that any action of the hormone in the maintenance of pregnancy is not exerted on the reactivity and motility of the uterine muscle.

Furthermore, the results do not support the view that gonadotropic hormones may in the guinea pig play a part in the control of uterine activity by a direct action on the muscle, since there is no evidence of any action on the uterine reactivity or motility with the hormone obtained from pregnancy urine. The doses of gonadotropic hormone used were greatly in excess of those necessary to produce quiescence of the uterus in the rabbit (Reynolds, 1932).

The experiments have revealed a discrepancy between the effective doses of oxytocin in vivo and in vitro. It is to be noted that the low reactivity in vivo applies both to the longitudinal and circular muscle. In the rat and in the rabbit previous observers (Knaus & Clark, 1925; Robson, 1935 and 1936) have obtained substantial agreement between the concentrations of drugs effective on the uterus in the intact animal and on the uterus suspended in physiological solution. The cause of the discrepancy in the guinea pig must remain at present unexplained; it may be that the reactivity to oxytocin of the guinea pig uterus in vitro is usually higher than that of the uteri of other animals, e.g. the rabbit and the mouse at similar stages of the sex cycle.

The results obtained from the ovariectomised untreated animals (Group 1) require further comment. It was expected that in these animals the uterus would be atrophied and would show only small rhythmic contractions, and such was the case in GP 34. In GP 35, on the other hand, the uterus on the tenth day after ovariectomy was still moderately large and exhibited marked contractions similar to those observed in the oestrin treated animals. There is evidence, however, that oestrin may be produced in sites other than the gonads; Zondek (1934) has shown, for example, that quite appreciable quantities of oestrin are still excreted in the urine of the gelding (about 0.3 p.c. of the amount in the urine of the stallion which is the richest natural

source of oestrin). Moreover, quite large quantities of oestrin have been extracted from certain organs, especially the suprarenals (Callow & Parkes, 1936). It appears possible, therefore, that the uterus in the ovariectomised guinea pig may be subjected to the influence of an oestrogenic substance derived from extragonadic sources.

Summary

Methods are described for recording the movements of the circular and longitudinal muscle of the uterus in vivo. One method using an optical system gives direct records with a high degree of magnification on a smoked drum.

Ovariectomised guinea pigs were injected with oestrin and then with progestin or with gonadotropic hormones. The reactivity to oxytocin and the spontaneous rhythmic activity of the uterus were then measured in the animal in vivo, and also on isolated strips.

An entire horn was used for in vivo experiments, while the other horn was cut in two portions for duplicate in vitro determinations.

Neither progestin nor gonadotropic hormones produced any desensitisation of the reactivity to oxytocin or inhibition of the motility of the uterus, in vitro or in vivo.

The reactivity to oxytocin in vitro was high in all cases. The reactivity to oxytocin in vivo was smaller and

frequently much smaller than in vitro.

The spontaneous rhythmic activity in vitro was small; this was shown to be due to the halving of the horns.

The relation of the findings to the mechanism involved in the maintenance of pregnancy is discussed.

The expenses of this research were defrayed by grants made by the Medical Research Council. I am indebted to Prof.A. J.Clark for hospitality and for many valuable suggestions.

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Chapter Seven

THE OXYTOCIN CONTENT OF THE FOETAL PITUITARY

From the Department of Physiology, The University, Bristol, and the Institute of Physiology, The University, Glasgow, 1934 and 1935.

It has been widely accepted, at least as a working hypothesis that the oxytocic hormone of the posterior lobe of the pituitary gland plays an important part in the process of parturition. Such a view has received strong support from the finding that the uterine muscle of the rabbit at parturition becomes highly reactive to this hormone. Nevertheless, the observation that apparently normal parturition can occur in certain species in the complete absence of the pituitary (see Chapter One) has cast some doubt on the supposition that the posterior lobe hormone is essential for the process. The possibility that this hormone may be elaborated in sites other than the pituitary gland must be excluded before these experiments can be finally accepted as proof that an oxytocic hormone is not concerned in causing contraction of the uterus at the end of pregnancy. The suggestion that oxytocin may be produced in the hypothalamus or even in regenerated hypophyseal tissue still awaits confirmation. There is another possibility, which has received little attention, that the necessary hormone may be secreted by the intact pituitary

of the foetus; it is quite conceivable that hormones produced there might reach the uterine muscle. In this way it might be said that the foetus could play some part in deciding when it was to be born.

The object of this investigation was, therefore, to determine the oxytocin content of the foetal pituitary and to compare it with the hormone content of the mature gland.

Methods

The whole pituitaries were removed from sheep and pigs immediately after slaughter and put at once into acetone to dehydrate. In the case of the very immature foetuses the pituitary gland with the hypothalamic area was cut out as it was found impossible to dissect out the pituitary only. The dried glands were powdered in a glass mortar and extracted with 0.25 p.c. acetic acid. After making up any loss due to evaporation the extract was filtered and the filtrate was stored in the refrigerator.

The method of drying with acetone and then grinding up in a mortar results in a very fine division of the gland and the yield of active principle is much higher than that obtained by the older methods of mincing. If the glands are put into acetone a few minutes after death before sufficient acidity develops then only a small amount of the oxytocic principle passes into the acetone, but if there is a delay then up to 8 p.c. of the oxytocic

activity may be lost. This amount of loss would not affect to an appreciable degree the results quoted in the present chapter. It is most important to extract the pituitary material with 0.25 p.c. acetic acid; the resulting extracts have a pH of 3.8 to 4.4 and are comparatively stable. Extracts made with distilled water may lose 50 p.c. of the total activity. These points are well discussed with examples and quotations from the literature in the important paper by Kamm, Aldrich, Grote, Rowe & Bugbee (1928). In the present experiments where a very small amount of tissue was being extracted only A.R. standard acetone and acetic acid were used in order to reduce as far as possible the likelihood of false positive results with the extremely unspecific guinea pig uterus.

The filtrate was assayed on the guinea pig uterus using Pituitrin (Parke, Davis & Co., kindly supplied by Dr.White) as the standard. The method used was that of Burn & Dale (1922). As this investigation involved a large number of assays it was essential to have a convenient apparatus so that the addition of test and standard extracts could be added in a regular timed sequence. A tin smith made me a galvanised tank 18 in. by 13 in. by 12 in. deep, with an outlet tap at one corner. A rectangular frame 18 in. by 8 in. high fitted into grooves on the short sides of the tank and carried two uterine baths and their reservoirs. Behind this two carbon filament 250 watt heating lamps and the usual toluene thermostat and a propellor (for mixing the water)

on a vertical shaft dipped into the tank. The glass uterine baths were of 60 c.c. capacity and their lower ends were connected by rubber tubing to a tap on the outside of the bath and then to a filter pump on a water tap. The reservoirs were made up of a spiral of glass tubing of somewhat more than 60 c.c. capacity, the lower end being connected to an aspirator containing Burn-Dale solution (modified Locke solution) which was kept on a shelf above the tank. The upper ends of the spiral reservoirs were turned over so that the fluid passing out flowed into the uterus baths. The rubber tubing from the aspirator to the reservoirs was interrupted by taps placed on the sides of the tank so that each bath could be filled independently.

When an assay was to be undertaken the tank was filled from the hot and cold water taps so that the level of water (at 37°C.) was about $\frac{3}{4}$ in. below the upper edge of the uterus baths. The heaters controlled by the thermostat in conjunction with an enclosed mercury switch (Isenthal) maintained the temperature thereafter. The taps from the aspirator were turned on and the Burn-Dale solution flowed from the spiral reservoirs (which had now been heated by the water in the tank) into the uterus baths. Air (from a filter pump mounted on an oil tin) was bubbled slowly through the Burn-Dale solution from an opening near the lower end of a glass tube dipping into uterus bath; the extreme end of the air tube was bent into a hook shape. The guinea pig was killed

by a blow on the head and the uterine horns were dissected out and quickly placed in the baths; the threads on the lower ends were looped over the glass hooks and the upper ends were tied to levers writing on smoked drums. The test solution was added to the bath from a 1 c.c. pipette. When the contraction which resulted had just passed its peak the tap to the exhaust pump was turned and the Burn-Dale solution was sucked out. The other tap from the aspirator was then turned and the bath was filled with fresh warm solution.

The advantages of this apparatus are that no dead spaces exist, all the solution is sucked off; and the spiral character of the reservoir ensures that when cold solution flows into the reservoir from the aspirator it is heated in the shortest possible time by the warm water in the tank - further, the fresh cold solution does not mix with the warmed up solution so that the uterine bath receives solution at the right temperature on refilling. In many designs of this apparatus the reservoirs are cylindrical in shape but I found that in this case the cold solution flowed through the reservoir in an axial stream and did not simply push the warm solution out into the bath, as a result the bath temperature after a change of solution was several degrees below 37°C. This fall of temperature causes a contraction of the uterus and increases the time for it to relax completely to its original base line and so lengthens the assay.

A typical tracing of the method of assay is given in Fig.1; "A" means that the test solution of the amount indicated above the tracing was added to the bath," W "means that the solution was changed at this point.

Results

These are given in Tables I. and II. In the case of the foetal sheep (Table I.) it will be seen that the oxytocin content of the gland is very small up to a foetal length of about 10 cm., thereafter it rises gradually till the body length is about 29 cm. This will be more readily apparent in the graph (Fig.2) which gives in addition the foetal body weight against the crown rump length (in solid squares). The latter data are given by Malan and Curson (1936). The period of gestation in the sheep is about 147 days; the last solid square in the graph refers to a two day old lamb.

The data for the foetal pig (Table II.), although less complete, suggest that the hormone content varies in a similar manner.

Fig. 1. See text.

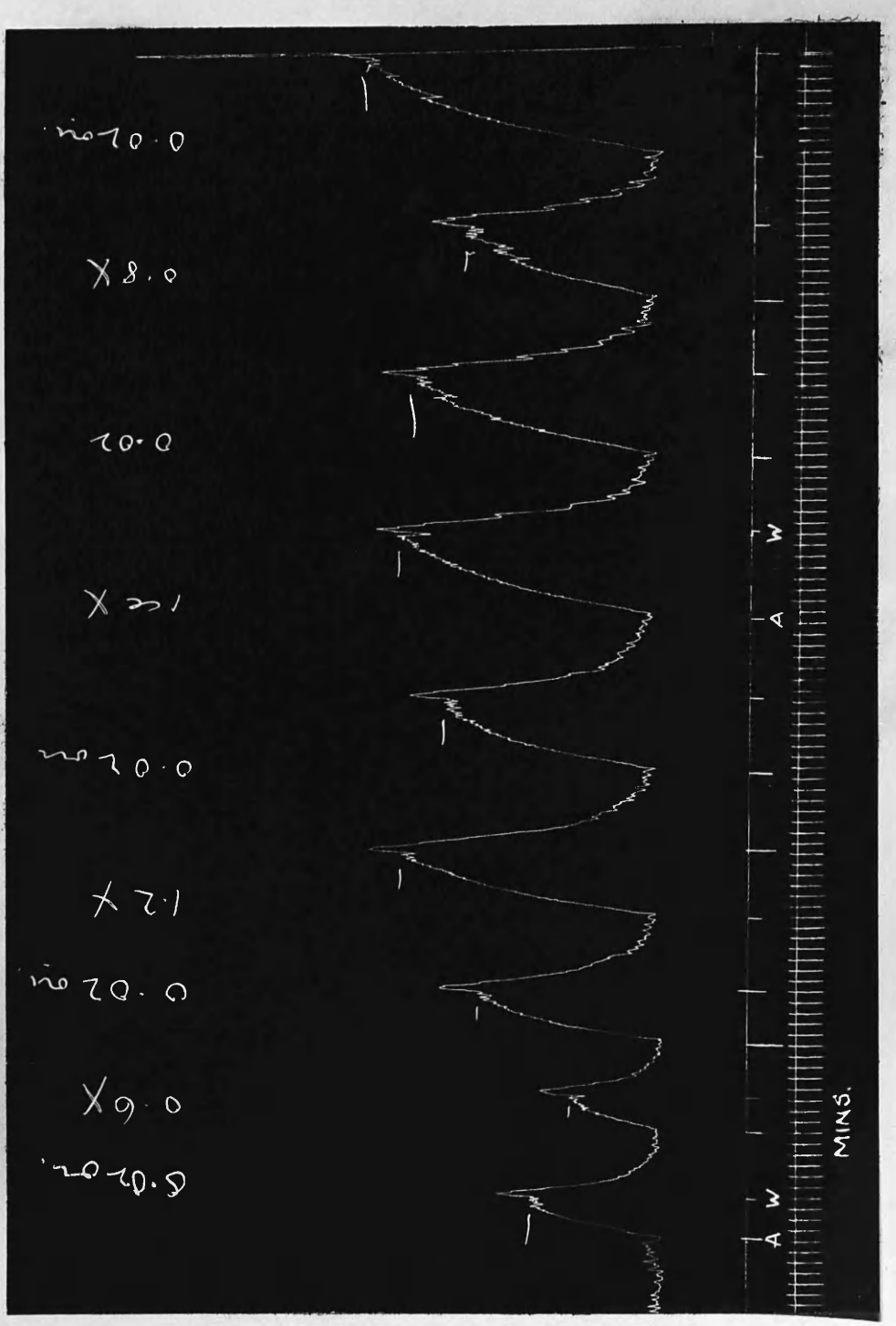

TABLE I.

Sheep No.	Foetal length cm.	No. of foetuses	Oxytocin content per foetal pituitary I.U.	Oxytocin content per adult pituitary I.U.
11	2.4	1	<0.02	
5	6.0	1	<0.02	
13	9.2	1	<0.06	
4	10.0	2	0.024	
12	12.8	1	<0.033	
8	17.5	3	<0.1	
14	18	1	0.032	
17	19	1	0.057	
3	20.5	1	<0.04	
26	21.5	1	0.075	
7	22.5	1	0.084	
9	22.5	1	<0.048	
25	24	4	0.08	
27	25.5	1	0.12	
16	26.5	3	0.082	
2	29	2	0.03	
28	29	1	0.2	
20	29.5	1	0.28	
18	30.5	1	0.25	
19	30.5	2	0.3	
21	31	1	0.27	
23	32	1	0.33	
6	34.5	1	0.091	
22	37	1	0.24	
24	37	1	0.18	
30	Adult ewe			7.0
31	Six adult ewes			7.25

TABLE II.

Pig No.	Foetal length, cm.	Foetal weight, g.	No. of foetuses	Oxytocin content per foetal pituitary I.U.
P6	12.1	96.1	20	0.002
P7	12.7	107.8	15	0.003
P5	12.9	112	9	0.003
P2	18.9	288	17	0.023
P9	22.5	642	13	0.12
P8	29.2	1168	17	0.039
P1	New born (stillborn)		1	0.27
R2	New born		4	0.9
R3	One day old		2	1.05
R6	Three days old		7	0.8
R4	Six days old		2	1.05

	Oxytocin content. I.U.
Adult sow	32
Adult sow	16
Adult sow	14

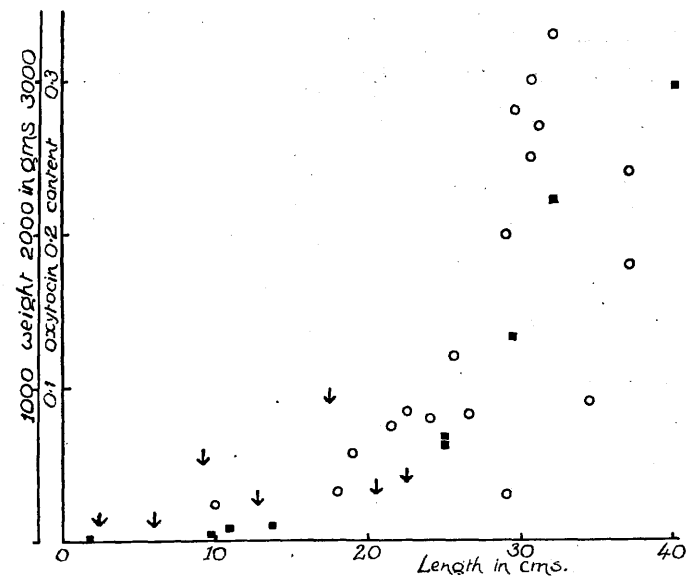

FIG. 2.—This presents the data of Table I. in graphical form. Abscissa, length of fœtus in cm. Ordinates, oxytocin content per pituitary in international units and weight of fœtus in g. The circles indicate the oxytocic content at the fœtal length on the abscissa. The down-pointing arrows indicate that the oxytocin content could not, in these cases, be exactly determined, but it was certainly below the value indicated by the base of the arrow. The solid squares indicate the length of the fœtus on the abscissa, which has the weight given on the ordinate.

DISCUSSION

The results of these experiments show that near parturition quite appreciable amounts of oxytocic hormone are found in the pituitaries of sheep and pig foetuses. Indeed in the case of the sow, which usually has a large litter, the total oxytocin content of the intrauterine pituitaries is of the same order as that of the maternal gland. In this species, therefore, the oxytocic hormone necessary for parturition might easily be provided by the uterine contents if it be assumed that the hormone can pass the placental barrier. That such an assumption is not an improbable one is suggested by the fact that insulin, which has also a fairly large molecule, appears to be capable of passing from the foetus into the maternal circulation.

In the case of the sheep which usually has one or two foetuses the difference between the oxytocin content of the intrauterine pituitaries and of the maternal pituitary is much larger - about ten to twenty times. In the absence of any information as to the rate of secretion of the pituitary gland as compared with the amount of hormone stored in the gland it is impossible to say whether adequate amounts of oxytocin could be supplied in the sheep by the foetal pituitaries. This possibility cannot, however, be excluded since there is good evidence that in certain glands, e.g. the suprarenal gland, the corpus

luteum, the testis, the amount of hormone stored is quite small as compared with the amount secreted.

Summary

The oxytocin content of the pituitaries of pigs and sheep at various stages of foetal life and in mature animals has been determined. Quite appreciable amounts are present in full term foetuses and the possibility that this hormone might pass into the maternal circulation and play a part in the process of parturition is discussed.

I am indebted to the following for pig and sheep pituitary material: Messrs.Spears Bacon Factory, Bristol, Mr.Syn of the Edinburgh Corporation abattoir, Messrs.R.D.Waddell, Glasgow, and Mr.Kent of Messrs.Irwin & Co., Glasgow. The expenses of the investigation were defrayed by a grant from the Medical Research Council.

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Appendix to Chapter Seven

The work just described suggested that it was possible that the foetal pituitary secretion might play a part in parturition if this secretion could pass the placental barrier. This possibility could best be tested out by injecting pitocin into an umbilical artery and observing whether or no the uterus contracted. This might be easily accomplished in the human case immediately post partum because the cord is long and large and may continue to function for some time after the birth of the child. I am not aware that this experiment has been performed; there are, of course, objections to inserting objects into the uterus to record its movements at this time - but these might be overcome by careful technique. This experiment is not possible in the lower animals because in most, if not all, the umbilical cord is broken at the birth of the young animal. I have made attempts to inject the umbilical arteries in guinea pig foetuses near term after making an incision in the uterus; but the cord is very short so that there is practically no space between the foetus and the placenta in which a needle on a syringe can be manipulated. The cord is also very fragile. I thought, however, that the matter could be tested in another, though less satisfactory, manner. A hypodermic needle had soldered on to it two cross arms about half way along its length. The following experiments were carried out on two guinea pigs near term. Under chloralose anaesthesia the uterus was connected to a lever by

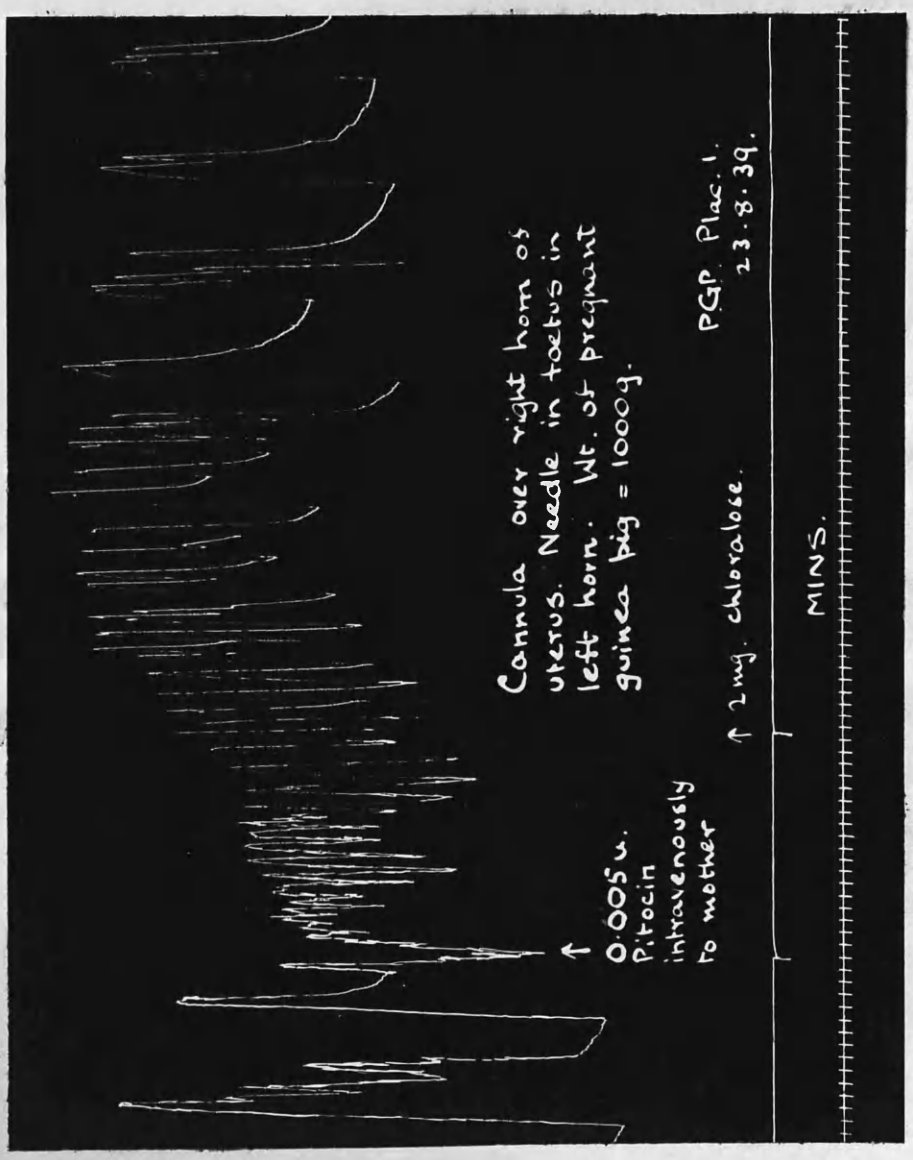

Fig. 3.

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means of the boat shaped cannula described in Chapter Six. A stab wound was made in the abdominal wall near a foetus in the other horn and the special hypodermic needle was passed through it and through the wall of the uterus into the abdominal cavity of a foetus; the cross arms of the needle were tied to the myometrium to prevent the needle from touching any maternal tissues. An injection of pitocin into the maternal jugular vein showed that the uterus could react to a small dose of oxytocin. Then increasing doses of pitocin were injected into the foetus through the special needle. Finally a dose equal to the largest injected into the foetus was given intramuscularly to the mother.

One of the experiments is illustrated in Fig.3. It will be seen that there was quite a marked reaction to 0.005 o.u. intravenously but that doses of oxytocin up to one unit into the foetus were without effect; one unit given intramuscularly to the mother produced a very marked and prolonged contraction. The results of the other experiment were very similar.

These experiments suggest then that oxytocin does not pass the placental barrier. One would imagine that 1 o.u. - if the placenta allowed diffusion from the foetal to the maternal circulation - would produce a very marked effect even allowing for the slowness of absorption from the tissues. There was only about two minutes delay between the intramuscular injection into the mother and the resulting uterine contraction. There is just the possibility, however, that the foetal circulation may have

been impaired or even brought to a standstill by the combined action of the anaesthetic and the injection of pitocin. In these circumstances a positive result would have been valuable and acceptable but a negative result is not a completely satisfying proof that the placental barrier is closed to oxytocin. It is indeed unfortunate that in every new attempt to advance further new and unexpected difficulties are met. But there is great value in scientific caution both for the sake of science and for the sake of one's own reputation.

Summary of Appendix

Oxytocin injected into a guinea pig foetus near term produced no effect on the mother's uterus in two experiments although it was shown that the uterus was very sensitive to oxytocin. This may mean that oxytocin cannot pass the placental barrier.

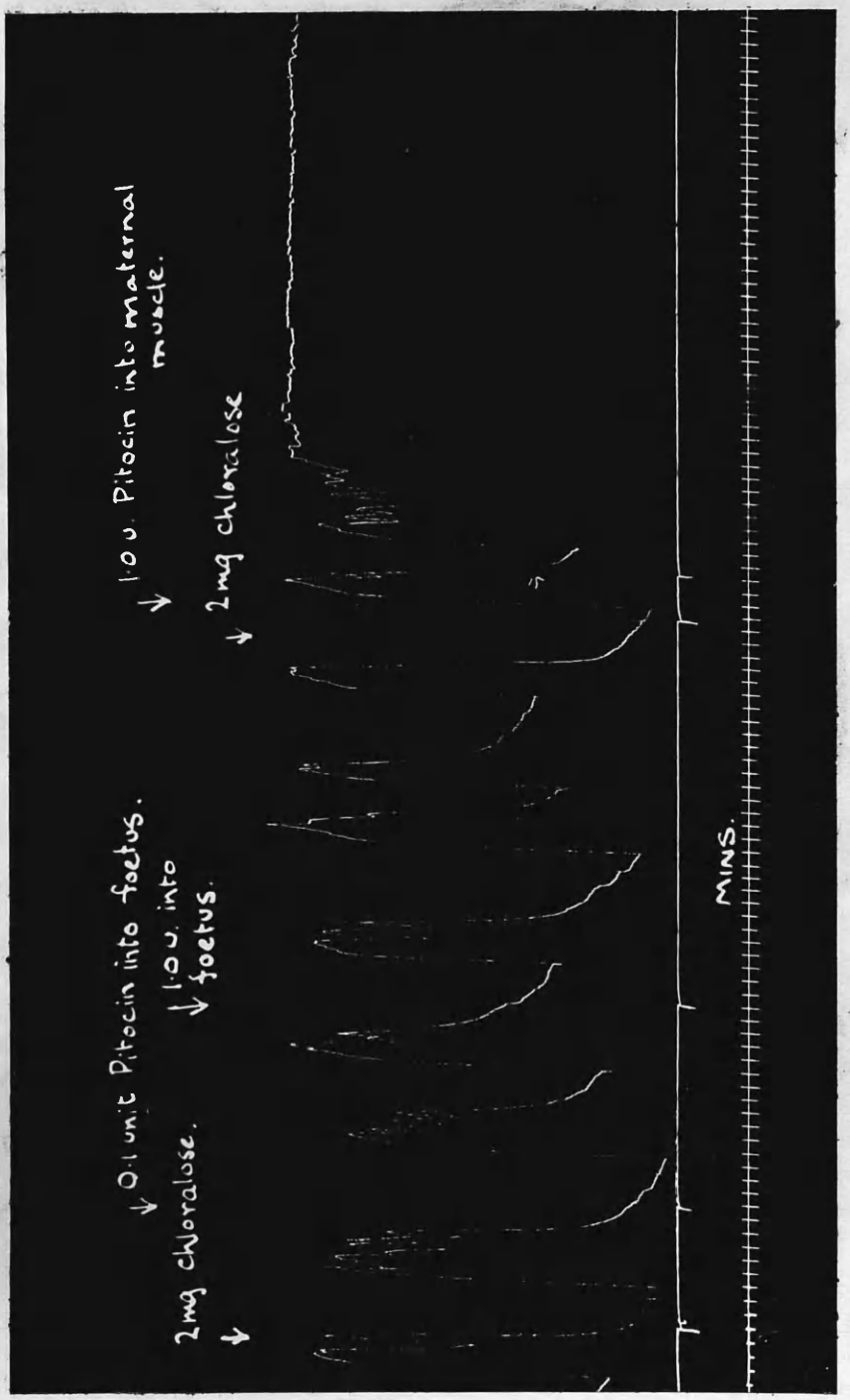
0·1 unit Pitocin into foetus.
2mg Chloralose.
1·0 u. into foetus.
1·0 u. Pitocin into maternal muscle.
2mg Chloralose
MINS.

Fig. 3.

Chapter Eight

THE EFFECT OF CERTAIN HORMONES ON THE ACTIVITY OF THE UTERINE MUSCLE OF THE MOUSE

From the Institute of Physiology, University of Glasgow, and the Department of Pharmacology, University of Edinburgh, 1936.

Previous investigations have shown that the ovarian hormones play an important part in determining the sensitivity (i.e. the reactivity to the oxytocic hormone of the posterior pituitary lobe) and the spontaneous activity of the rabbit's uterus. The effects on sensitivity can be demonstrated both on the isolated uterus and on the intact animal. Experiments on some other species have failed to give similar results. In the guinea pig, for example, the sensitivity and motility are not appreciably affected by these hormones (see Chapter Six).

Accordingly it seemed of interest to investigate the conditions in one of the smaller rodents to see how far the guinea pig or the rabbit is representative of the mammals. The experiments were performed on the mouse since data were available for the action of the ovarian hormones on the isolated uterus of this animal. (Robson, 1934a). In particular it appeared necessary to determine whether oestrin would increase the sensitivity in vivo as it is known to do in vitro, and whether progesterone would show any effect on the motility and sensitivity as investigated in the intact animal. At the same time a number of experiments were done with testosterone since it has been shown that

this hormone will inhibit the oestrous cycle in mice (Robson, 1936) and also that it can produce desensitisation of the rabbit's uterus both in vitro and in vivo (Robson, 1937).

Methods

The experiments were performed on mature mice. All the animals were ovariectomized by the dorsal route, and the uterine contractions were recorded seven days afterwards. The animals can be divided into four groups: (1) a control group of three uninjected mice; (2) six animals injected with oestrone only; (3) ten animals injected with oestrone and progesterone; (4) five animals injected with oestrone and testosterone.

The oestrone (which was administered to all animals except group 1) was injected in oily solution in six doses on the morning and evening of the third, fourth and fifth days after ovariectomy, the total amount in each case being 20 microgrammes.

Progesterone was given in oily solution in six doses on the morning and evening of the fourth, fifth and sixth days after spaying, the total amount administered being 0.75 mg. in each of five mice, 1.5 mg. in each of two mice, and 3 mg. in each of three mice.

A total amount of 3 mg. of testosterone in oily solution was given to the mice of group 4. The hormone was administered in six doses on the morning and evening of the fourth,

fifth, and sixth days after ovariectomy.

I am indebted to Messrs.Schering & Co. for the supply of progesterone in oil (Prolution), to Dr.Miescher of Ciba Ltd. for the testosterone, and to Dr.White of Parke, Davis and Co. for a sample of specially purified oxytocic hormone (purified Pitocin).

The animals were anaesthetized with chloralose (6 to 7 mgm. in saline subcutaneously). The movements of the longitudinal muscle of the uterus were magnified by a photoelectric method and recorded on smoked paper by the method previously described for the guinea pig (Chapter Six). The uterus was attached to the cannula and lever, and the abdomen was closed. The body temperature was read by means of a thermocouple inserted with the cannula into the abdomen. By varying the distance between a reading lamp and a layer of cotton wool covering the animal the temperature could be kept steady at 37°C. Recording of uterine contractions was not begun until the temperature was within physiological limits. By maintaining the body temperature in this way the preparation could be kept in apparently good condition for five hours or more. These small animals can be very quickly heated up or cooled down - their weights varied between 25 and 39g., or about 1 oz. - and although at first the thermocouple was regarded as a refinement it was soon recognised as an essential part of the apparatus.

Oxytocin was injected in a constant volume of 0.1 c.c.

saline into a tail vein. In all experiments an attempt was made to estimate the smallest amount of oxytocin necessary to elicit a definite motor effect. This was taken as a measure of the sensitivity of the uterine muscle.

Vaginal smears were taken from all animals just before the recording of the contractions.

Results

The results are given in Table I. The following abbreviations are used to describe the cells found in the vaginal smears:- C = cornified cells; E-C = cells in transition from epithelial to cornified; L = leucocytes. The spontaneous activity is expressed as the actual movement in millimetres of the centre of the uterus.

It will be seen that the spontaneous activity was small in group 1, it was higher in groups 2, 3, and 4, but there was no appreciable difference between these three groups in this respect. It can, therefore, be concluded that administration of oestrone increases the motility of the uterus but that neither progesterone nor testosterone produces any inhibitory action.

The sensitivity (i.e. reactivity to oxytocin) in the last three groups was greater than in the untreated ovariectomized animals of the first group. No significant difference in sensitivity between the animals of the last three groups could be recognised. It must, therefore, be concluded that treatment with

TABLE I.

Group	Animal No. MO.	Weight gms.	Vaginal smear	State of uterus	Treatment: Oestrone microg.	Treatment: Progesterone mg.	Treatment: Testosterone mg.	Spontaneous activity mm.	Reactivity to oxytocin in international u.
1.	5	26	-	Small	-			0.9	>0.01 <0.03
	7	26	-	Small	-			0.1	>0.02
	7A	26	-	Small	-			0.2	>0.15
2.	3	26	C	Oestrous	20			1.3	<0.01
	4	27	C	Oestrous	20			1.9	>0.0005 <0.002
	8	27	C	Oestrous	20			1.2	<0.01
	10	33	C	Enlarged, not distended	20			6.1	-
	16	35	C	Oestrous	20			6.0	<0.01
	16A	30	C	Enlarged, some distension	20			5.7	>0.0005 <0.002
3.	6	28	C	Enlarged,not distended	20	0.75		1.2	>0.01 <0.03
	9	25	C	Enlarged, not distended	20	0.75		3.0	-
	9A	-	C	Enlarged, partly distended	20	0.75		3.5	<0.002
	11	35	C	Enlarged, not distended	20	0.75		8.0	-
	11A	35	C,E-C,L	Enlarged, not distended	20	0.75		8.0	0.005
	14	30	E-C,C,L	Enlarged, not distended	20	1.5		8.0	>0.005 <0.01
	14A	32	C,E-C,L	Enlarged not distended	20	1.5		5.0	0.01
	17A	37	C,L	Enlarged, not distended	20	3.0		3.4	<0.003
	17B	39	C,E-C,L	Enlarged, not distended	20	3.0		6.0	<0.01
	18B	35	C,E-C,L	Enlarged, not distended	20	3.0		4.3	>0.001 <0.003
4.	12	29	C	Enlarged, not distended	20		3.0	2.4	0.003
	12A	27	C	Oestrous	20		3.0	5.8	0.001
	13	29.5	C	Enlarged, partly distended	20		3.0	5.7	>0.001 <0.003
	13A	35	C,L,E-C	Enlarged not distended	20		3.0	8.0	>0.01
	15	32	C	-	20		3.0	6.7	>0.003 <0.01

oestrone increases the sensitivity of the uterine muscle to oxytocin but that neither progesterone nor testosterone cause any desensitisation.

The dose of oestrone chosen was sufficient to produce full vaginal cornification in all animals of group 2; four of these animals showed full oestrous distension of the uterus and the organ was partly distended in a fifth animal. Partial inhibition of the vaginal reaction to oestrone was produced by progesterone in six out of the ten mice of group 3. In no animal of this group was a full oestrous distension of the uterus seen.

Representative tracings are given in Fig.1 and Fig.2, from an oestrin treated and a progestin treated mouse respectively. In spite of the small amount of uterine muscle it was possible to obtain tracings which filled the kymograph drum.

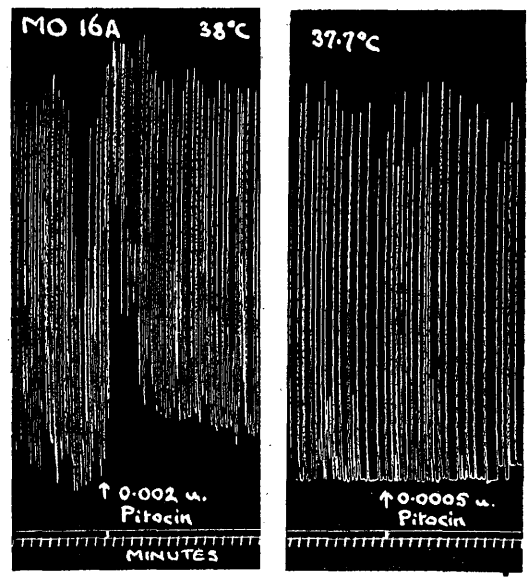

Fig. 1. Record of movements *in vivo* of the longitudinal muscle of the uterus of mouse, which had been injected with œstrone only. Response to injection of 0·002 unit of "pitocin" intravenously but not to 0·0005 unit. The average movement of the centre of the uterus was about 5·7 mm. The temperatures indicate the abdominal temperature shown by the thermocouple. Time intervals in minutes. There was an interval of 30 min. between the two parts of this tracing.

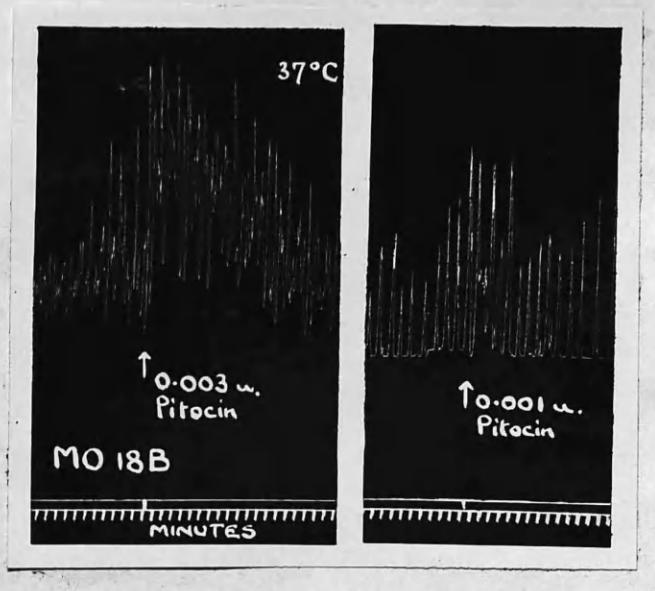

Fig. 2. Record of movements in vivo of the longitudinal muscle of the uterus of mouse MO 18B, which had been injected with oestrone and progesterone. Response to 0·003 units of Pitocin intravenously but not to 0·001 unit. The average movement of the centre of the uterus was 4·3 mm. Time intervals, 1 min. There was an interval of 54 mins. between the two parts of this tracing.

DISCUSSION

In these experiments an attempt has been made to use the various hormones in such dosage as is needed to produce the changes occurring in the normal physiological cycle. Hence the mice were injected with 20 microgrammes of oestrone since this amount (i.e. about 200 units) must be given to bring about the full changes of oestrus (Marrian & Parkes, 1930). Progesterone was given in amounts up to 1 mg. per day. This may seem a large dose, but since about 1 mg. per day is necessary to maintain pregnancy in an ovariectomized mouse (Robson, unpublished data) it must be considered to be within physiological limits. That the dose of testosterone administered was more than adequate is suggested by the fact that much smaller daily amounts will inhibit the oestrous cycle in mice (Robson, 1936).

The previous investigations on the rabbit (Robson, 1934b) have suggested that the alterations in the sensitivity of the uterine muscle during pregnancy and parturition can be explained in terms of the action of the two ovarian hormones, oestrin and progestin. Experiments on the uterus of the mouse in vitro have shown that the oestrous hormone increases the uterine sensitivity as in the rabbit (Robson, 1934a) and the present investigations demonstrate that this also holds for the uterus in the intact animal. Progestin does not desensitize the uterus of the mouse in vitro and this is not due to any discrepancy between the behaviour of the muscle in saline and the

intact animal as it is now shown that no desensitisation occurs when the uterus is examined in vivo.

For comparison with the data of other chapters, it is of interest to note that the doses of oxytocin necessary to produce contraction of the uterus of the mouse in vitro and in vivo are of the same order, as is borne out by the following considerations. The uterus of the oestrin treated mouse will react in vitro to a dose of about 0.02 unit per 100 c.c. (Robson, 1934a); smaller doses are effective only when the oestrin treatment has been very intensive. In vivo the injection of 0.001 unit may be effective in causing a contraction of the uterus. This represents a concentration of 0.04 unit per 100 c.c. on the assumption that the blood volume is 2.5 c.c. A marked discrepancy between the doses of oxytocin necessary to cause contraction of the uterus in vitro and in vivo such as has been found in the guinea pig (Chapter Six) is thus not observed in the oestrin treated mouse.

The effects of the ovarian hormones and of testosterone on the sensitivity (i.e. response to oxytocin) of the uterine muscle in various species has been summarised up to 1937 in Table II. (Rabbit: Knaus, 1930, Robson. 1933a, Makepeace, Corner & Allen, 1936. Guinea pig: Siegmund & Kammerhuber, 1931, work described in Chapter Six. Mouse: Siegmund, 1930c, Robson, 1934a, work described in the present Chapter. Rat: Siegmund, 1930 a, b, c. Cat: Robson & Schild, personal

communication. Bitch: Robson & Henderson, 1936. In the case of the human, the results of Knaus, 1931, at first suggested that progestin desensitised the uterus but later observers, e.g. Moir, 1934, have not confirmed this).

TABLE II.

The effect of various hormones on the response to oxytocin of the uterine muscle of various species - taken from the literature up to 1937.

Species	Action on sensitivity to oxytocin						Sensitivity to oxytocin at parturition	
	Oestrin		Progestin		Testosterone			
	In vitro	In vivo	In vitro	In vivo	In vitro	In vivo	In vitro	In vivo
Rabbit	x	x	-	-	-	-	xxx	xxx
Guinea Pig	x	0	0	0			xxx	
Mouse	x	x	0	0		0	xxx	
Rat			0					
Cat	-	0	0	?	x	?	xxx	xxx
Bitch	-		0					
Human				?			xxx	xxx

x means increase.

\- means diminution.

0 means no effect.

? means either that no very definite effect has been observed, or that (in the human case) the matter has not been finally decided between various workers.

The data contained in Table II. emphasise the remarkable diversity of the effects of these hormones on uterine sensitivity. Nevertheless the fact remains that in the four species (rabbit, mouse, cat, guinea pig) that have been so far quantitatively investigated the sensitivity of the uterine muscle increases during pregnancy and reaches a maximum at parturition. Brooksby (1937) has shown that the response of the rat's uterus in vitro to oxytocin does not increase up to the twentieth day of pregnancy - unfortunately no observations on the condition at parturition were made. In some of these species the increase of sensitivity can be adequately explained by the action of the two ovarian hormones, although only in one (i.e. the rabbit) is the luteal hormones known to produce any effect on the sensitivity of the uterus to oxytocin. Consideration of the action of the ovarian hormones on the motility of the uterus in different species reveals divergencies and difficulties of a similar kind (see Reynolds, 1937).

In view of the variations of the effects produced by these hormones the possibility must be considered that the same end effect on the sensitivity of the uterus at parturition may be brought about in different species by different hormonal mechanisms. This is a discouraging conclusion to arrive at in view of the hope raised in Chapter One that this fundamental process would admit of a general explanation with modification in details only for various species.

Summary of Chapter Eight

The activity of the uterine muscle of the mouse has been studied in vivo in four groups of animals:-

1. Ovariectomized and uninjected.
2. Ovariectomized, then injected with oestrone.
3. Ovariectomized, then treated with oestrone and progesterone.
4. Ovariectomized and injected with oestrone and testosterone.

In the first group the reactivity to oxytocin (i.e. sensitivity) and the spontaneous activity were small. In the last three groups the sensitivity and the spontaneous activity were greater than in the first group, but there was no appreciable difference between these three groups in these respects. Neither progesterone nor testosterone has any modifying effect on the activity of the oestrone - treated uterus.

There is no difference between the sensitivity in vitro and in vivo of the uterus of the mouse treated with oestrone.

The effects of the ovarian hormones and of testosterone on the sensitivity of the uteri of various species have been tabulated and the problems involved in explaining the variations of sensitivity and of motility during pregnancy and parturition are discussed.

The expenses of this research were defrayed by a grant made by the Medical Research Council. I was again indebted to Prof.A.J.Clark for hospitality.

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Chapter Nine

THE HUMAN FOETAL ELECTROCARDIOGRAM

From the Institute of Physiology and the Department of Obstetrics, University of Glasgow, 1937.

It has been my habit to enter into a small book from time to time what are facetiously termed bright ideas. This particular idea remained nothing more than a note for several years before I could construct the necessary apparatus. To those interested in electrical methods there is considerable fascination in the problem of recording potentials due to the activity of the foetal heart; the first paper on the subject appeared only a few years after Einthoven had described his string galvanometer which was to remain for long the most sensitive apparatus for this kind of work. Since this first paper by Cremer in 1906 there have been only about a dozen more, a fact which would suggest that the efforts of the workers in this field had not been greatly rewarded. It is quite certain that, if an electrocardiogram of the foetus could be obtained with ease and certainty, it would quickly become a routine procedure. A foetal electrocardiographic tracing would provide an immediate diagnosis of pregnancy without the delay associated with the present very useful biological methods; it might provide an indication of the foetal condition during a prolonged labour and might give an early indication of foetal death; it might also

give some indication of the position of the child in the uterus; it might be of value in multiple pregnancies and in deciding how many of the foetuses were alive. These are some of the advantages which will accrue on the invention of a successful method. This happy state has by no means been reached and it seems to me that quite novel methods will have to be invoked for its solution - the difficulties will become apparent later in this chapter. Recently the technique of recording the very small potential differences encountered in nerves has been greatly improved chiefly by the Cambridge physiologists; I thought, therefore, that it would be worth while to re-investigate this subject as I would have a considerable advantage over the earlier workers. This accounts then for what may be called an electrical interlude in these investigations of reproductive physiology.

Cremer (1906) was the first to claim to have made an electrocardiographic tracing of the foetal heart. He used an abdominal electrode in combination with either a vaginal or a rectal electrode and obtained very small but regularly recurring deflections which were independent of the maternal deflections. Foà (1911) using much the same method obtained in three cases dips in the electrocardiographic tracing which occurred at a rate similar to that of the child's heart and were, therefore, supposedly foetal in origin. Neither of these papers is very

convincing. Sachs in 1922 tried to obtain a foetal electrocardiogram using all sorts of leads in several patients with no success. Small deflections of the curve which might be attributed to the foetal heart disappeared completely on hypnosis. He used the double coil galvanometer of Siemens-Halske.
v. Haynal & Kellner (1924) used electrodes placed one on the abdominal wall over the fundus of the uterus and the other over the symphysis pubis. They obtained deflections which coincided with foetal movements and smaller deflections which resembled the original foetal deflections described by Cremer. But they were unable to state categorically that these deflections originated in the foetus.

In contrast to this very doubtful success in the human subject is the early success of the veterinarians who were dealing with larger foetuses and probably with larger heart potentials. Nörr in 1921 experimented on pregnant mares. He saturated the hair with a solution of zinc sulphate and applied amalgamated zinc electrodes. The ordinary leads (I., II. and III. of Einthoven) gave no foetal deflections but when he brought the electrodes much nearer the foetus, e.g. one on each flank, then foetal deflections appeared - the rate being about 86-100 per minute as compared with the maternal 50-56 per minute. There seems little doubt that Nörr obtained deflections which were of foetal origin. It is interesting that no report of experiments

on the pregnant cow has been found. Before the work on the human subject to be described below was begun I attempted (as a good physiologist ought) to obtain foetal electrocardiograms in the guinea pig and the cow - in both cases without success. The cow presented an unexpected difficulty because the hide seemed to be a most excellent insulator; even if the hair was clipped off and the skin soaked in saline it was impossible to obtain any maternal deflections with skin leads - electrocardiograms were obtained, however, when one electrode was clipped on to the nasal septum and the other was placed in the rectum. It was, therefore, very unlikely that any foetal deflections would be obtained and the attempt was soon abandoned. This preliminary work on the cow was carried out at the Hannah Dairy Research Institute. Curiously enough Nörr (1921a) published an account of the electrocardiogram of the cow and did not mention any such difficulty. It may be a peculiarity of the Ayrshire breed.

A very short report on the human foetal electrocardiogram was given by Maekawa & Toyoshima in 1930. They used a thermionic valve amplifier with a string galvanometer but they did not state the sensitivity of the apparatus. Electrodes were applied to the mother's abdominal wall over the head and buttocks of the child. As far as one can see - for the tracings are very poorly reproduced - these are genuine foetal electrocardiograms; but there are no controls and no time marks. In 1933 Steffan &

Strassmann had some success with a thermionic valve outfit which was more sensitive than the string galvanometer. They used Leads II. and III. and also abdominal electrodes placed as nearly as possible on the long axis of the foetus. There is no doubt that they obtained genuine foetal electrocardiograms - but the reader is asked to examine the tracings with a lens. Strassman published the same work (with the same illustrations) from the Mayo Clinic in 1936 but added very little to the original description; he made other reports in 1938 and 1939 without adding anything of consequence. Johnson (1938) reported the accidental finding of foetal deflections in one case in Leads II., III and IV.F. These deflections were very tiny and not easily distinguishable in the reproduction and the author said that they must be taken on trust as they are certainly present in the original photographs.

Method

The Institute of Physiology did not possess an electrocardiograph till about 1932 when I constructed a valve amplifier using the rather primitive valves and apparatus available at that time. At first this amplifier was not very reliable but with increasing experience and improvement in valves and circuit details the apparatus by 1936 had become very reliable indeed and now this kind of apparatus gives routine satisfaction. The function of the amplifier is to magnify the potentials available from the

heart so that finally sufficient current of the same wave form can be obtained to work a relatively insensitive but very robust oscillograph of very high natural frequency. It is particularly in this last respect that the newer oscillographs score over the string galvanometer which has a natural frequency of say 300 cycles per second as compared with at least 1000 cycles per second for a moving iron oscillograph. The principles underlying the construction of a high quality moving iron oscillograph had been given by Matthews (1928) but, as I discovered later, certain important details were omitted so that it was not possible from his description to make an oscillograph which was as satisfactory as the Matthews oscillograph made by the Clifton Instrument Company in which Matthews was a director. However, with the help of our mechanic, Mr.A.R.Smellie, I constructed an oscillograph of the Matthews pattern out of loudspeaker parts and an old ergometer magnet. It was used for class purposes before being applied to the present problem.

A recording camera had next to be constructed. Not long after the setting up of the British Broadcasting Company about 1922 pictures and diagrams were transmitted several times a week by the fultograph system which was fundamentally not very different from the present method of transmitting pictures to newspaper offices. After a few years the Company decided that there was no general interest in the pictures and the transmissions ceased. The fultograph outfits were then sold off for about thirty

shillings. The clockwork device for rotating the drum through an electric clutch formed an excellent basis for the construction of an electrocardiograph camera; the drum carrying the bromide recording paper (Ilford Recorder) was placed behind a cylindrical lens obtained from Barr and Stroud. When a button was pressed, the electric clutch carried the drum round once only, at the same time a shutter opened and then closed to prevent fogging of the paper.

A phonic wheel driven by a tuning fork as supplied in the Cambridge Instrument Company's electrocardiograph was also constructed and worked well although it required considerable attention. It was found more convenient and sufficiently accurate to use a 2/9d. alarm clock with a shortened hair spring; a very light lever attached to the escapement wheel interrupted the light falling on the camera and produced the wavy line seen in the figures. The hair spring was adjusted till the distance between the peaks was exactly one fifth of a second by comparison with a 100 cycles per second tuning fork photographed at the same time on the camera.

The apparatus used in this research was thus decidedly "home made" and was not all that might have been desired. But it cost less than £20 whereas commercially made apparatus would certainly have cost over £200. I have a certain amount of doubt now as to the wisdom of such economy because it usually occurs at the expense of the valuable time of the experimenter. The

great virtue of home constructed apparatus is that one has no scruples about making even radical alterations and these are very often required in the quite unpredictable course of research work.

The four valve amplifier originally constructed had a sensitivity three times greater than that of the standard string galvanometer - i.e. its sensitivity was 3 cm. per millivolt. It was soon found that this sensitivity would be most unlikely to produce useful results. Increasing the sensitivity of the apparatus is not a matter simply of more valves and batteries. Ordinary audiofrequency amplifiers in gramophones rarely have more than three stages of amplification because of the difficulty of avoiding self oscillation with resulting distortion and also because it is difficult to avoid picking up so called interference. Fortunately the problem of interference had been solved to a large extent by Matthews (1934) with his balanced input amplifier which consists of two valves in a bridge circuit. Interference from lighting or power electric circuits picked up by the patient acting as a kind of aerial is carried to the grids of the two valves in phase and does not alter the output of the amplifier but potentials originating in the patient make one grid less negative and the other grid more negative and in this case a magnified voltage appears across the output resistances. When this circuit was rigged up and added to the amplifier it was soon found that the movements of the base line as the results of maternal movements or sudden foetal kicks were so great that

the amplifier was frequently paralysed for several seconds and the light was as frequently off the camera as on it. It was obvious that for this purpose the coupling condensers recommended by Matthews were too large; they were reduced from 4 microfarads to 0.1 and finally to 0.02 microfarad. This reduction of the time constant of the amplifier allowed the galvanometer to return quickly to zero after a large deflection evoked by an involuntary movement of the mother or of the foetus, or by the greatly enlarged maternal R wave. Another advantage of using small coupling condensers was that the interval between the R waves became isoelectric because the slow P and T waves were not amplified; this allowed the small foetal deflections to be more easily detected. Even with this alteration the base line showed fairly rapid random oscillations due partly to electrical causes (battery and valve "noise") but chiefly to action potentials arising apparently from the muscles of the mother's abdomen. The latter were greatly increased if the patient was uncomfortable or nervous. If the foetal deflections are less than these random variations they cannot be detected.

It is of interest to enquire into the alteration of amplitude produced by the alteration of the time constant of the amplifier. In a condenser coupled valve amplifier the voltage produced by the first valve is applied to the coupling condenser C and the grid resistance R (of the second valve) in series.

Only the voltage across the grid resistance R is applied to the second valve. If C is one microfarad and R is one megohm then the time constant is one, i.e. any charge acquired by C will fall to 0.37 of its initial value in 1 second; but if the value of C is only 0.02 microfarad then the charge will fall to 0.37 of its initial value in 0.02 second. The recovery of the amplifier is thus very quick after a large impulse. The following table shows how the amplification of the lower frequencies is affected by the alteration in the coupling condenser. The figures in the two right hand columns give the percentage of the theoretical amplification which will be reached at the frequencies given in the left hand column. Thus one can easily see that the very slow P and T waves of the electrocardiogram are not amplified when small coupling condensers are used and they do not appear in any of the tracings given here except in the case of the children after birth (Fig.3) when the 1 mfd. coupling condensers of the original electrocardiographic amplifier were used.

Frequency in cycles per second	C = 1 mfd. R = 1 megohm.	C = 0.02 mfd. R = 1 megohm.
10	99	80
1	99	12
0.5	95	2.5
0.1	52	1.3

Leads. It was most important to determine as soon as possible the most favourable position for leading off to the amplifier. The best results were obtained with one electrode on the mother's abdomen over the fundus of the uterus and the other over the symphysis pubis. The electrodes were metal discs about two inches in diameter covered with two layers of gauze soaked in saline. Non-polarisable electrodes are not required where the input resistance of the amplifier is over 50,000 ohms as the current produced by one millivolt is insignificant. The input resistance of the balanced input amplifier is 400,000 ohms. With a condenser coupled amplifier skin currents are not amplified and no compensation is required. The electrodes were held in position by broad elastic bands; or, more conveniently, by a nurse wearing rubber gloves. A vaginal electrode was not tried as most of the cases were near term; in any case if the method were to prove useful enough to be employed in a routine manner it would be rather inconvenient to have to use a vaginal lead. A rectum-fundus lead was tried out but proved to be unsatisfactory. The discomfort produced by the rectal electrode resulted in movements of the rectal sphincter which greatly disturbed the base line. The superiority of the longitudinal lead (fundus-symphysis pubis) over the other leads tried, namely, fundus-rectum, bilateral (lumbar-lumbar) and anteroposterior (umbilicus-lumbar vertebrae) may be due to the

fact that in the longitudinal lead neither electrode is very near voluntary muscle owing to the divarication of the recti during the later months of pregnancy. The base line is undoubtedly least disturbed in the longitudinal lead.

Results

Fig.1 shows an electrocardiogram obtained under the most favourable circumstances. The large waves are, of course, the maternal R waves; the smaller waves indicated by the arrows are assumed to be foetal deflections. These are of the order of 25 to 50 microvolts. As the moving iron oscillograph used did not give deflections exactly proportional to the applied voltage, exact measurements of voltage cannot be made. The white line at the end of the tracing indicates the deflection produced by 100 microvolts.

The foetal heart rates measured on the photographs are within normal limits; but as the foetal heart rate is continuously varying the figure calculated from the photograph did not necessarily agree with that obtained by previous auscultation of the foetal heart. The waves are regularly spaced however, and most unlike any that might be produced by interference from electrical machinery or by irregularities in the battery supply. This successful result was obtained in only one third of the cases which have, therefore, been classified

as "positive".

In the great majority of cases the foetal deflections were much smaller. In one third the deflections were scarcely greater than the irregularities of the base line, and they could be picked out only with great difficulty. These cases are classified as "doubtful". The remainder of the cases showed no deflections of a foetal character and are classified as "negative". Fig.2 shows two tracings, the upper of which is classified as doubtful and the lower of which is negative. It should be explained that as the foetal heart was heard in both instances there is no doubt that the foetus was alive at the time of examination.

Two cases of twin pregnancy are included in the series; only one yielded foetal deflections. This case, illustrated in Fig.3, is of special interest. It will be seen that the two deflections are slightly different in shape; the upper row of strokes points to the sharper deflections, the lower row to the slightly broader deflections. The deflections are quite regularly spaced; that is, during the time of the photograph, the two hearts were beating quite regularly but at slightly different rates which are shown on the photograph. So far as I know, this is still a unique record. The foetal heart rates seem to vary quite independently; the slower heart in the first tracing became the faster heart in the second tracing taken a few minutes later. An X-ray photograph confirmed the finding that

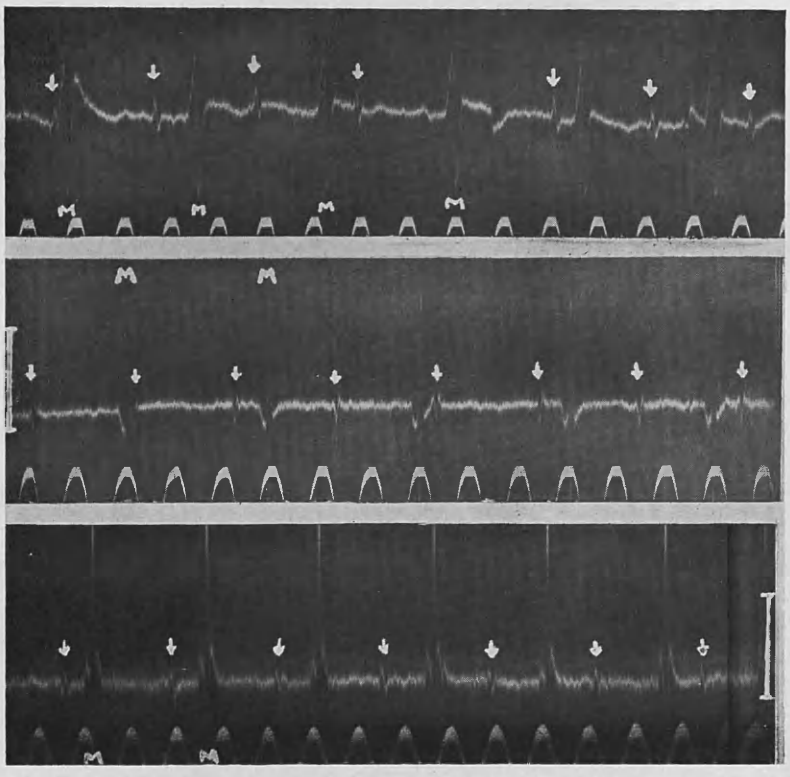

FIG. 1.

Upper tracing, July 16th, 1937. Mrs. F., age 24 years, primigravida. Male child, vertex presentation, 7 days before delivery; 0.1 mfd. coupling condensers; foetal heart-rate from tracing, 143 per minute.

Middle tracing, July 29th, 1937. M.B., age 24 years, primigravida. Male child, vertex presentation, 6 days before delivery; 0.02 mfd. condensers; foetal heart-rate from tracing, 145 per minute.

Lower tracing, December 30th, 1937. Mrs. H., age 22 years, primigravida. Male child, vertex presentation, delivered 2 days later; 0.02 mfd. condensers; foetal heart-rate from tracing, 151 per minute.

In all cases arrows indicate the foetal deflections. The time is in fifths of a second. *M* indicates the maternal deflections. The deflection for 0.1 millivolt is shown.

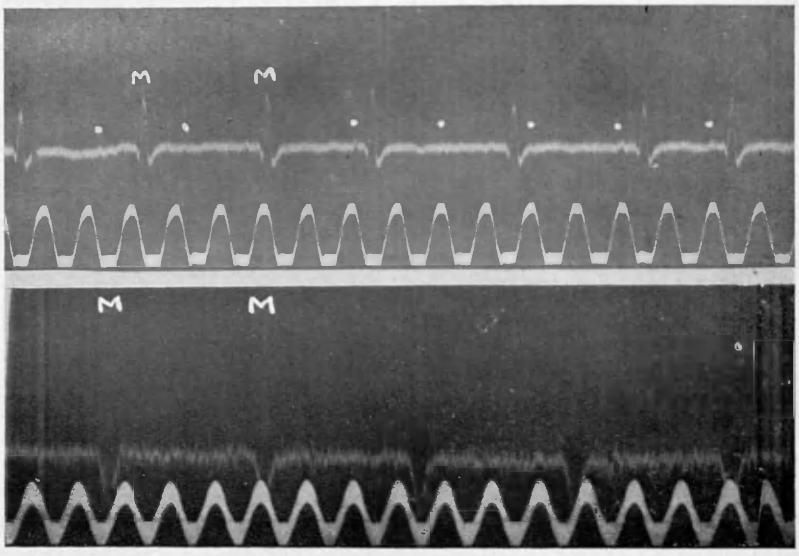

FIG. 2.

Upper tracing, August 18th, 1937. Mrs. McT., age 31 years, primigravida. Male child, vertex; delivered September 27th, 1937. Dots have been placed above deflections which are regular and may be foetal. Classed as doubtful.

Lower tracing, December 7th, 1937. Mrs. MacD., age 29 years, 2-para. Female child, vertex; delivered next day. No deflection of foetal character. Classed as negative. Sensitivity and time as in Fig. 1.

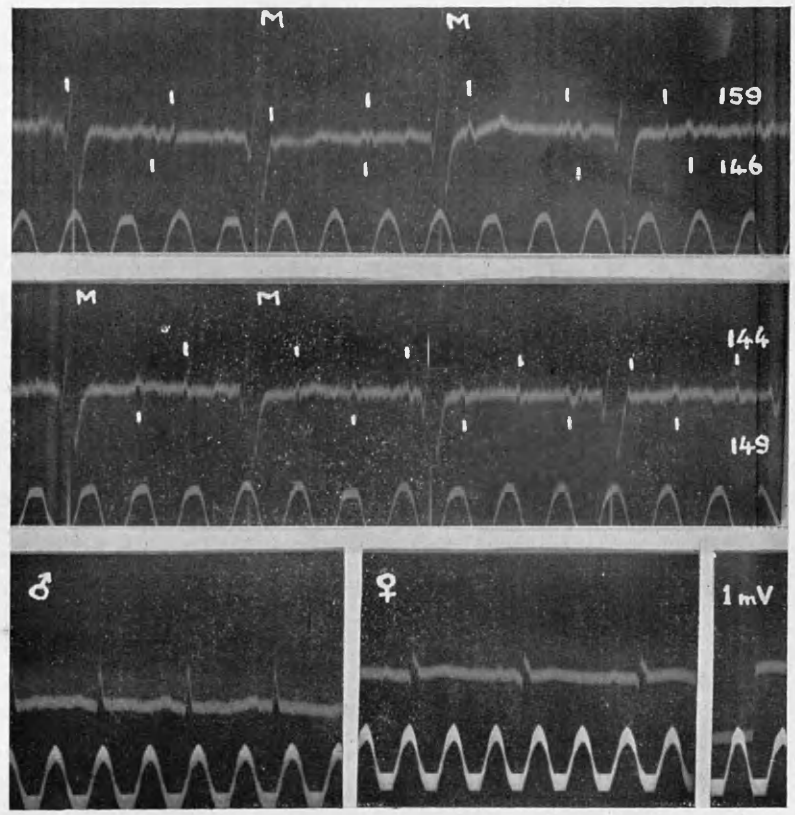

FIG. 3.

Mrs. S., November 26th, 1937, age 27 years, 5-para. Twins, both breech, one male, one female, delivered December 30th, 1937. Hydramnios, abdominal circumference 42 inches. Sensitivity and time as in Fig. 1.

The vertical strokes point to the foetal deflections; the top row to the sharper deflections (possibly male) and the lower row to the broader deflections (possibly female). The figures indicate the foetal heart-rate calculated from the photograph. The lower tracings were obtained from the two children 7 days after birth; on the left the male and on the right the female. The deflection produced by one millivolt is also shown.

the two foetuses were breech presentations, with one lying somewhat obliquely. The foetal deflections are in the same direction as the main maternal deflections. Inspection of Fig.1 will show that in the vertex presentation the foetal and maternal deflections are in opposite directions; this is explained by the fact that in this presentation the two hearts, maternal and foetal, have their apices pointing in opposite directions. This finding plus the finding that in a twin pregnancy two sets of deflections are obtained form the best proofs that the deflections are actually foetal in origin. A completely satisfying proof could only be given by simultaneous records of foetal heart sounds and foetal electrocardiograms.

In the lower part of Fig.3 are shown the electrocardiograms of the twins one week after birth together with the deflection for 1 millivolt. This interval of one week was unavoidable as it was not thought wise to move them up to the electrocardiographic room immediately after birth. In order to make these records comparable with the antenatal ones they were obtained by using leads from electrodes on the vertex and buttocks. There is quite obvious splintering of the QRS complex in the case of the female which distinguishes it clearly from that of the male. It is very tempting to compare these R waves with those obtained before birth and to say that the deflections marked by the upper row of strokes are due to the male child

and the other set to the female child. The fact that the deflections are of slightly shorter duration in the antenatal curves is readily explained by the fact that the coupling condensers in the antenatal experiment were 0.02 mfd. while they were 1.0 mfd. in the other case. As the two condensers in the balanced input amplifier are in series this means a difference in the time constant of 100 times. As there is some evidence, however, that the form of the electrocardiogram in foetal and postnatal life may not be the same (Smith, 1922) it would not be wise to lay too much stress on the similarity of the antenatal and postnatal R waves of Fig.3.

Steffan and Strassmann (1933) suggested that it would probably be found best to use leading off positions in the electrical axis of the foetal heart. In Fig.3 the two deflections are of the same size in spite of the obliquity of one of the foetuses. It thus seems unlikely that variations of the foetal cardiac axis would lead to difficulties in obtaining a foetal electrocardiogram.

DISCUSSION

Electrocardiograms were taken from 35 mothers, all of whom except two were within two months of full term. As these two cases yielded results classed as negative and because they were 4 and 4½ months from term they are omitted from the remainder of this discussion. The majority of the remaining 33 cases were admitted to the wards because of contracted pelvis and all were well enough to walk to the electrocardiographic room. For the purposes of these experiments they can be regarded as normal. The foetus was certainly alive at the time of examination in 30 cases, and was probably alive in the remaining 3.

Of the 33 cases, 10 gave results classed as positive, 11 gave doubtful results, and 12 gave negative results. It cannot be said, therefore, that the method has at present much clinical value, especially as the foetal heart can usually be heard without difficulty in the last two months of pregnancy. It can at least be said in its favour that, unlike so many biological tests for pregnancy, it would not be expected to produce any false positives.

The earliest occasion on which a positive result was obtained was 34 days before delivery (case of Fig.3). As only four cases were examined at a greater distance from term one cannot say with any degree of certainty whether this represents

the earliest possible time at which a positive result is obtainable.

The rather limited success which attended this investigation suggests that either the proper technique has not yet been found or that for some reasons connected with the electrical properties of the mother or of the child the foetal potentials are not available at the surface.

Regarding the first suggestion it is at present very difficult to see in which directions the technique could be altered with advantage. Greater amplification would be difficult to obtain without the use of screening; in any case if the foetal potentials are less than the electrical oscillations of the base line no amount of amplification will reveal them. Apart from this, the irregular disturbances of the base line due to electrical potentials arising in the mother's abdominal wall may obscure the foetal electrocardiogram; if this is the case no degree of amplification, large or small, would render them detectable. If the patient is excitable or apprehensive then the prevailing disturbance level is high. Any method of reducing the irregularities of the base line would presumably lead to a larger number of positive results. Sachs (1923) in attempting to obtain a foetal electrocardiogram found that little waves on the graph which at first were suspected to be foetal disappeared under hypnosis. It is doubtful whether it

would be justifiable to submit patients to hypnosis or to a general anaesthetic as a routine measure.

Turning now to the second suggestion, it will be obvious that if the heart of the foetus is producing only a small action potential then it will be extremely difficult to pick it up on the surface. Low voltage electrocardiograms are quite well known in postnatal life. It is very difficult to think of any method of finding the intrauterine potential. Smith (1922) has shown that immediately after birth and before breathing commences the electrocardiogram shows left sided preponderance while once breathing has commenced there is a right sided preponderance. In his cases the potential of the R wave was not small. Bearing this limitation in mind it might be supposed as a starting point for discussion that it is likely that the postnatal voltage of the R wave would bear some direct relation to antenatal voltage of the R wave. The electrocardiograms of 30 children were, therefore, obtained within a few days after birth using electrodes placed on the vertex and the buttocks to make the lead as similar as possible to the antenatal one. The average value of the R wave was found to be about 1 millivolt. The smallest voltage found was that of the R wave of the female child illustrated in Fig.3, yet her R wave was seen antenatally. This is by no means conclusive but it at least points to the notion that negative results are not due to

deficiency of the R wave of the child.

The average weight at birth of the children giving positive results was 7 lb. 8 oz. with a standard deviation of 8.7 oz.; the average weight of the children giving doubtful and negative results was 7 lb. 4 oz. with a standard deviation of 18.9 oz. The difference of the means is 4 oz. but this cannot be regarded as significant as the standard error is 4.8 oz. The actual size of the child and, therefore, of the heart would not appear to influence the results.

In the positive group there were 8 male foetuses and 3 female; in the other groups taken together the ratio of male to female foetuses was 4.7 to 3. It is most unlikely that the sex of the child plays any part because in the case illustrated in Fig.3 where a male and a female child were present in the same uterus the potentials generated by both appeared at the surface.

As it seems difficult to obtain evidence against the foetus let us examine in turn the layers surrounding it. Theoretically it might be suspected that the best conditions of the surrounding layers expressed in electrical terms would be a high radial conductivity and a low spherical conductivity. We might expect then that excess of liquor amnii would prevent the potentials reaching the surface at sufficient intensity. In one case examined after a "considerable amount" of liquor had escaped after spontaneous rupture of the membranes about 24 hours before

delivery the result is classed as doubtful in spite of the finding postnatally of a relatively large R wave. In two of the cases classed as positive there was an excess of liquor amnii - one was that of Fig.3. These findings suggest that the amount of liquor is not an important factor. This is very difficult to understand. Some preliminary experiments (Bell and Sheehan, unpublished) of another investigation on the rabbit with electrodes pushed through the uterus on either side of a foetus showed incidentally that it is easy to obtain a foetal electrocardiogram when the foetus is lifted out of the saline bath in which the mother is immersed but difficult when it is returned to the bath.

It is not easy to make any measurements of the other coverings of the foetus. After the first 10 cases had shown that positive results were not invariably obtained, the abdominal circumference at the umbilicus was measured in all cases. In the four measured cases in which positive results were got, the average abdominal circumference was 34.7 in. with a standard deviation of 0.9 in. (omitting the twin pregnancy as it is not comparable); in the doubtful and negative cases measured, the average circumference was 36.2 in. with a standard deviation of 4.3 in. (again omitting a twin pregnancy). The difference of the means is 1.5 in. with a standard error of 1.1 in. This is therefore not highly significant but the difference is in the direction which fits in with the rest of the argument and may be true enough

although the tests of significance are not fulfilled in the small group. If we assume that the increase of circumference was brought about mainly by an increase of thickness of the anterior abdominal wall and to a negligible extent by an increase posteriorly - which seems a reasonable assumption - then the abdominal wall was on the average $\frac{1}{2}$ in. thicker in the unsuccessful cases.

It is well known that as a general rule the abdominal wall becomes thicker with advancing age and with increasing parity. Of the positive results 7 were obtained in primiparae, three in multiparae (1 2-para, 1 3-para, 1 5-para); of the remainder of the cases (doubtful and negative) eight were primiparae, and fifteen were multiparae (9 2-parae, 2 3-parae, 1 4-para, 3 5-para and above). The average age of the positive group was 27.1 years; the average age of the other cases was 30.4 years.

Since all these three attempts to assess the importance of the abdominal wall point in the same direction one feels that there is reasonable ground for concluding that the electrical properties of the abdominal wall are important. This may be summed up by saying that in a young primipara with a thin abdominal wall there is a very great likelihood that a foetal electrocardiogram will be obtained.

SUMMARY

Using a thermionic valve electrocardiograph capable of very high amplification the electrocardiograms obtained when leads are taken from the abdomen of pregnant women show in some cases waves which are almost certainly foetal in origin. The evidence supporting the foetal origin is that the direction of the foetal deflection depends on the presentation (vertex or breech) and in a case of a twin pregnancy two sets of deflections were obtained.

Of 33 cases examined in the last two months of pregnancy about one third showed positive results, but the other two thirds showed no wave on the electrocardiogram which could be definitely recognised as foetal.

It is suggested that the failure to secure positive results in all cases is due partly to differences in the electrical properties of the abdominal wall, and partly to the electrical disturbances produced by the abdominal muscles, and not to insufficient sensitivity of the apparatus or to differences between the foetuses.

ACKNOWLEDGMENTS

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Chapter Ten

THE MOVEMENTS OF THE UNLOADED UTERUS

From the Institute of Physiology,
University of Glasgow, 1936-8.

This is in some respects the most important piece of work to be described in this thesis. It yielded results which were completely unexpected and quite at variance with accepted opinion founded on the results of many hundreds of experiments. It removed a strange anomaly of uterine behaviour, but at the same time it made the explanation of the maintenance of pregnancy in the rabbit more difficult, that is, as difficult as in other species.

It is well known that the activity of the smooth muscle of the intestine is greatly modified by tension within the lumen, but the effect of tension on the smooth muscle of the uterus has not received so much attention. It has been shown by Newton (1933) that the activity of the pregnant guinea pig uterus in vitro is considerably modified by altering the load on the recording lever, and also that the threshold dose of oxytocin varies with the loading. It would not be wise, however, to apply these findings to the behaviour of the uterus in vivo; direct comparison of the activity of the uterus of the pregnant guinea pig in vivo and in vitro shows that the behaviour of the

same piece of uterus is quite different under the two conditions (see Chapter Twelve). So far as is known, no method of recording the uterine contractions in vivo has been described in which the uterus is subject neither to tension nor to distension. The pressure used in many experiments, especially in the human experiments discussed in Chapter One, to distend the uterine balloon is very high indeed. An accidental finding is described by Allen & Reynolds (1935) thus: "In the course of an experiment the intrauterine balloon ... became bulged, and increased in capacity ... the contractions became much greater in this case while in the other experiments of this series the uteri became quiescent". Reynolds reviewing the subject in 1937 said: "Further consideration ought to be given, in the future, to the characteristics which various procedures impart to the kymographic records of uterine activity". The experiments which will be described in this chapter were designed to eliminate tension and to investigate the movements of the uterus under conditions as far as possible normal.

Methods

The method briefly was to take serial X-ray photographs of wire stitches placed alongside the uterus. The method seems first to have been used by Barcroft in his important work on the spleen (See Barcroft, Harris, Orahovats & Weiss, 1925). Very

similar methods were used later by McSwiney during his work on gastric motility in dogs and by Westman on tubal movements in the human subject. These workers took only a few photographs of each experiment; the present work seems to be the first to have required a kind of cinematographic series.

The rabbit was chosen as the first experimental animal because it is relatively large and because modifications of its activity are well known and well defined. The abdomen was opened under aseptic conditions and small stitches of 36 s.w.g. silver wire were sewn at short intervals along the mesometrial border of one horn of the uterus from the vaginal end to the junction with the uterine tube. The stitches were inserted in the mesometrium about 2 mm. from the uterus to avoid direct irritation of that organ, and in the spaces between the blood vessels to minimise interference with the blood supply. From five to eight stitches were used and each was tied in a distinctive fashion (e.g. once through and tied, once through and tied twice, twice through and tied once, and so on) so that the order could be recorded at the operation and recognised later in the photographs of which Fig.1 is an example. It will be readily appreciated from this picture that the stitches were sufficiently distinctive in appearance and that they were very small indeed and not likely to interfere with the animal in any way; indeed most of the animals put on weight and in the process the mesometrium became filled with fat which

almost hid the stitches from view at the final examination. This filling up of the mesometrium is a perfectly normal occurrence in the rabbit comparable to the filling up of the mesentery with fat in human beings. In two experiments there was an adhesion between one of the stitches and the bladder, but no adhesion to intestine was ever found. This is an important part of the argument as to the cause of the movements observed at the time of the experiment. No reaction round the stitches was seen at the post mortem examination even when the stitches had been in position for several months; the absence of inflammatory changes was confirmed by histological examination in all cases. Inflammatory changes have been shown by Laufer & Reynolds (1938) to produce uterine motility.

As the serial X-ray photographs had to be taken without disturbing the animal an aluminium topped operating table was altered so that a brass frame running in a groove could be slid under the thin aluminium top. The bottom of the tray was made of thick lead so that the X-rays would be absorbed and thus the definition would be improved by reduction in secondary radiation. When the tray was greased it moved easily in and out and caused no apparent disturbance of the animal. The unanaesthetised rabbit was fastened in the prone position to the table and if there was no undue noise it remained perfectly quiet except at the points indicated on the graphs. At the time of the experiment

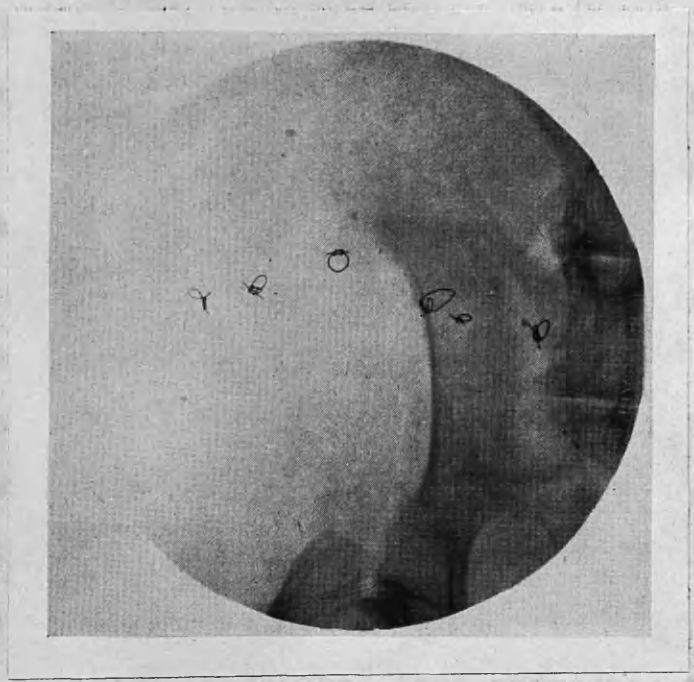

Fig. 1. Positive from rabbit X 6 (actual size) to show the appearance of the stitches.

the tray was gently pulled out, a double wrapped film ($4\frac{1}{4}$ x $3\frac{1}{4}$ in.) placed on it and the tray slid gently back into position beneath the animal. An X-ray photograph of the stitches was then taken (exposure time 2 sec. except in X 6 where it was 1/20th.second; the tube film distance was 24 in.). This process was repeated every half minute during the course of the experiment. Oxytocin (Pitocin kindly supplied by Dr.White of Parke, Davis & Co.) was given into an ear vein in 1 c.c. of saline towards the end of the experiment. Finally the animal was killed and specimens of the uterus taken for histological examination.

It must not be imagined that this piece of work was done with the latest chromium plated marvel of X-ray apparatus. The source of high tension current for the tube was an old fashioned coil with a rotary interrupter which gave a rather poor output and required therefore, long exposures for very thin objects. The tube was fortunately a Coolidge tube although the focus was rather broad. All this was apparatus discarded from various hospitals. Very soon after the beginning of the experiment the tube was nearly white hot but yet it seemed to carry on.

To facilitate sorting, serial numbers were painted on the outer wrappings of the films with white lead paint; as an additional check this number was written (in the dark room) in pencil on the film immediately before development. The distances

between the shadows of consecutive stitches in each film were measured by dividers and tabulated; the most easily recognised point in each stitch was chosen as the reference point. This was an extremely tedious job as each of the graphs has about 500 measurements plotted in it. From the tables the graphs were constructed as follows:- the length of the first interstitch distance at the vaginal end of the uterus was plotted vertically above the time axis, and each interstitch distance was added in succession, so that the total ordinate represented the total length of the uterus at that instant - as the uterus was usually curved the total length on the graph was greater than the distance between the shadows of the first and last stitches. Each line on the graphs describes the movements of one stitch. In addition to these movements of contraction and relaxation other movements were seen, for example the whole uterus might move up or down in the abdomen or the curve of the uterus might alter considerably. It is very difficult to know how to reproduce these alterations in a diagram; the only way would be to project them as a cinematographic sequence. This has not been done.

Results

Rabbit X 2 was a normal, intact, presumably oestrous animal; it was used for a first try-out of the method. The uterine movements (Fig.2) were recorded two weeks after the insertion of the stitches. In considering Fig.2 and the subsequent graphs

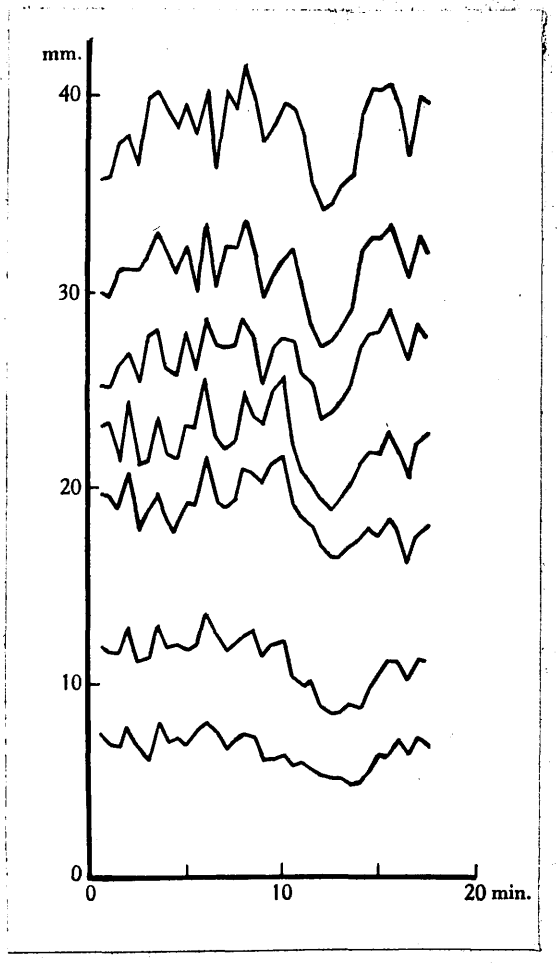

Fig. 2. Rabbit X 2, presumably oestrous, two weeks after insertion of silver stitches.

several points must be borne in mind. The method gives practically no magnification but, for convenience, the measurements have been plotted on a slightly enlarged scale; small alterations on the graphs are, therefore, of little moment. It was usually possible to measure the interstitch distance to 0.2 mm., but occasionally the error might be of the order of 0.5 mm. In the case of X 2 with seven distances to be measured it is just possible, if accidentally the errors summed, for the values on the top curve to be, say, 3 mm. in error. It will be seen that the dip in the topmost curve which begins about 10 minutes after the beginning of the experiment is over 6 mm. in depth - this is undoubtedly a genuine contraction. Further the smooth curve of this contraction strongly suggests that theerror of measurement is, in fact, quite small. Not very much stress can be laid on the smaller alterations of the curve - for example from 4 to 10 minutes in this case.

There were some struggling movements of the animal at 11 minutes but the contraction started at 10 minutes. It has frequently been noted in other experiments where uterine movements were being recorded directly on a drum that a uterine contraction may produce restlessness even in an anaesthetised animal. When, as in the present case, the animal is conscious struggling might be expected more frequently. Since contractions are associated fairly often with struggles, but a struggle is not

necessarily accompanied by a contraction, it is probable that the uterine movement is the primary occurrence. It is difficult to imagine that any of the uterine movements illustrated here were painful - that is, if human experience is of any value as an analogy.

Rabbit X 3 was spayed and silver stitches were inserted along the left uterine horn; one week later photographs were taken. The curve (left hand of Fig.3) shows a preliminary relaxation followed by a very slight contraction. The early relaxation is of the same extent as the contraction seen in Fig.2, but generally speaking the uterus is less motile. A small piece of uterus taken from the right horn showed histologically the typical spayed appearance. (Fig. 8, top left). An adhesion between the vaginal stitch and the wall of the bladder was broken down. Beginning on the next day subcutaneous injections of 0.01 mg. oestrone in olive oil were given twice daily for one week (total dose 0.14 mg.). On the day after the last injection another series of photographs yielded the second graph of Fig.3. The difference between the two graphs is striking: the oestrin treatment has increased the movements of the uterus and has increased the total length (comparing the lengths at maximum relaxation). Injection of 0.02 unit of pitocin was followed by a contraction which may have been spontaneous. This dose would almost certainly have produced a marked effect had the usual

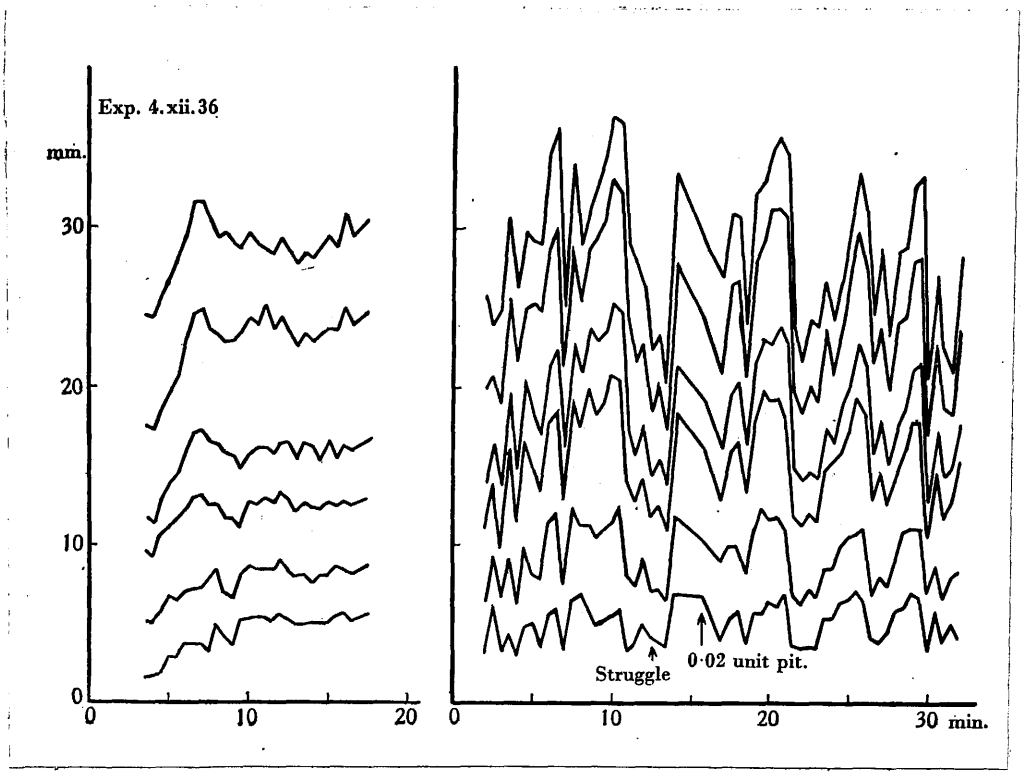

Fig. 3. Rabbit X 3 spayed 27 November 1936. Left hand graph obtained 4 December 1936. Then injected with oestrone 4 - 11 December 1936. Right hand graph obtained on 12 December 1936.

methods of recording been used. (For histology see Fig.8, top right).

Sixteen days after the silver stitches had been inserted rabbit X 4 received intravenously 4.6 mg. of mare anterior pituitary powder in suspension in saline. This powder was prepared by dropping mare anterior pituitaries into acetone as soon as possible after the animal was killed at Pinkston; after a change of acetone (the first acetone contains a great deal of fat which interferes with the next process) the acetone was removed in a vacuum dessicator; the dry pituitary was then ground up in a glass mortar. The X-ray photographs (Fig.4) were taken nine days later, which is about or just after the height of pseudopregnancy; this was confirmed by the very marked progestational proliferation seen on histological examination of the uterus. (Fig.8, bottom left) The great length as compared with X 2 and X 3 is very obvious. The most striking point is the great extent of the spontaneous movements.

Numerous investigators, beginning with Reynolds & Friedman (1930), have reported that the rabbit uterus in pseudopregnancy is quiescent. In the case of X 4 the uterus was at one time less than half its maximum relaxed length. The spontaneous contractions are separated by long quiescent periods of ten to fifteen minutes; this is in great contrast to the oestrin-treated rabbit (Fig.3) where contraction wave followed contraction wave

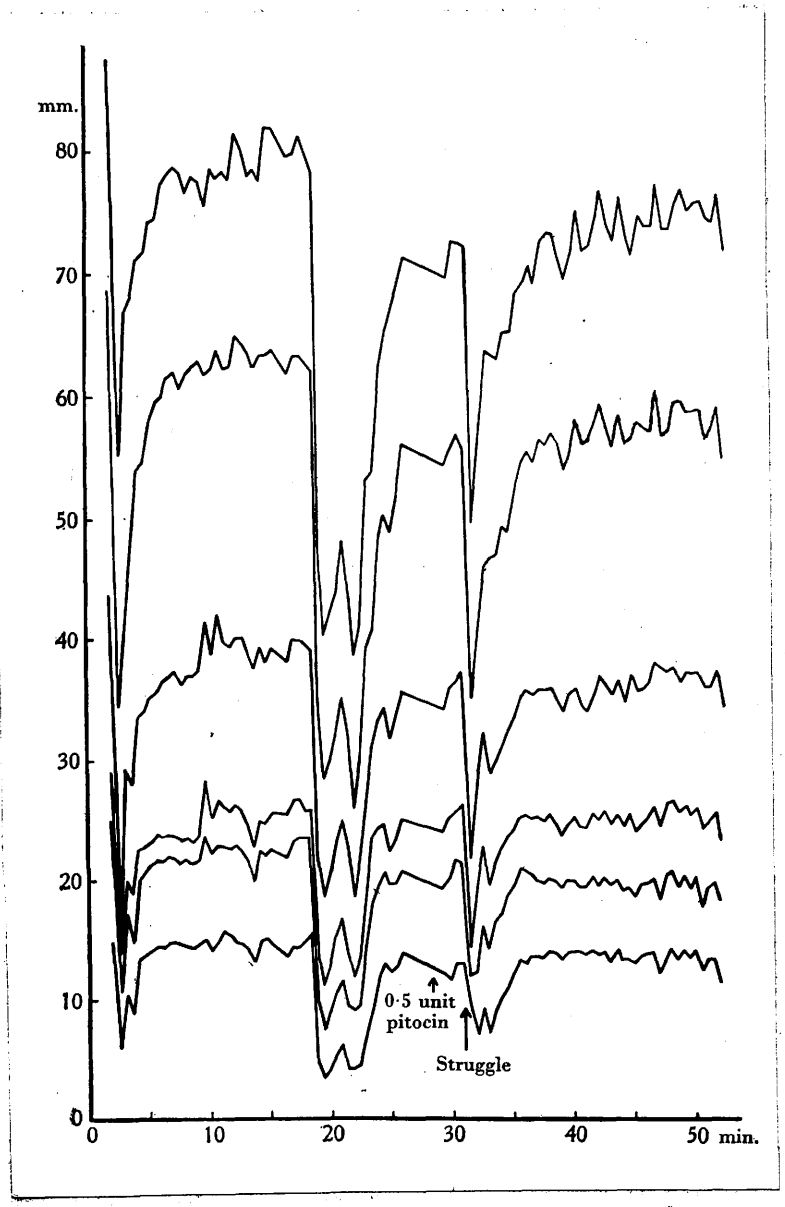

Fig. 4. Rabbit X 4. Stitches inserted 1 April 1937. Mare pituitary powder injected 17 July 1937. Experiment on 26 July 1937.

without intermission. Injection of 0.5 unit of pitocin at 28 minutes did not appear to have any effect; some time was lost in making this injection - this is indicated on the graph by the straight lines from 26 to 29 minutes.

As experiment X 4 showed such a great difference from the generally accepted state of affairs it was decided to repeat the experiment in a slightly different way. Rabbits X 5 and X 6 were oöphorectomised and stitches were inserted in the left mesometrium. About four weeks later each was given a course of oestrone (0.01 mg. per day for a week) followed by 0.25 mg. progesterone (Proluton, kindly supplied by Schering Ltd.) twice daily for four days. The photographs were taken on the day after the last injection. Fig. 5a is the graph of X 5 and Fig. 5b the graph of X 6. It will be seen that these curves are essentially similar and not very different from Fig.4; again there are long periods of quiescence followed by marked contractions. The uteri of both X 5 and X 6 showed marked progestational proliferation (see Fig.8 bottom right) but they were not quite so long (i.e. by comparison of lengths at maximum relaxation) as in the case of X 4, presumably because the latter had nine days' progesterone treatment from its own ovaries as compared with four days' injection treatment in X 5 and X 6. In X 5 injection of 0.5 unit of pitocin was followed by a contraction of about five minutes duration which may have been spontaneous; but the contraction is more sustained thar the

two spontaneous ones; in X 6 injection of 0.5 unit of pitocin was, unfortunately, given shortly after the beginning of a contraction which was smaller than the previous spontaneous ones. This absence of response to relatively large doses of oxytocin is also seen when ordinary recording methods are used. The findings in the three different states of the uterus - after spaying, after oestrin treatment, and in pseudopregnancy - are very well contrasted in Fig.5c which shows the top lines only of three of the graphs given already in complete detail. The three graphs of Fig.5c are drawn to the same scale.

Discussion

One of the apparent disadvantages of this method is that the detail of the curves is not so great as can be obtained by ordinary kymographic procedures. If the curves are examined closely it will be found that the omission of every other point would have altered very little the general trend; this simple test shows that the photographs were taken frequently enough. Increasing the number of photographs in a given time would have added very greatly to the cost of the experiment, and it would have increased the labour of measurement by requiring several hundred additional measurements for each graph without yielding any corresponding advantage.

The possible causes of the movements of the stitches are respiratory, intestinal and uterine. The outlines of the stitches

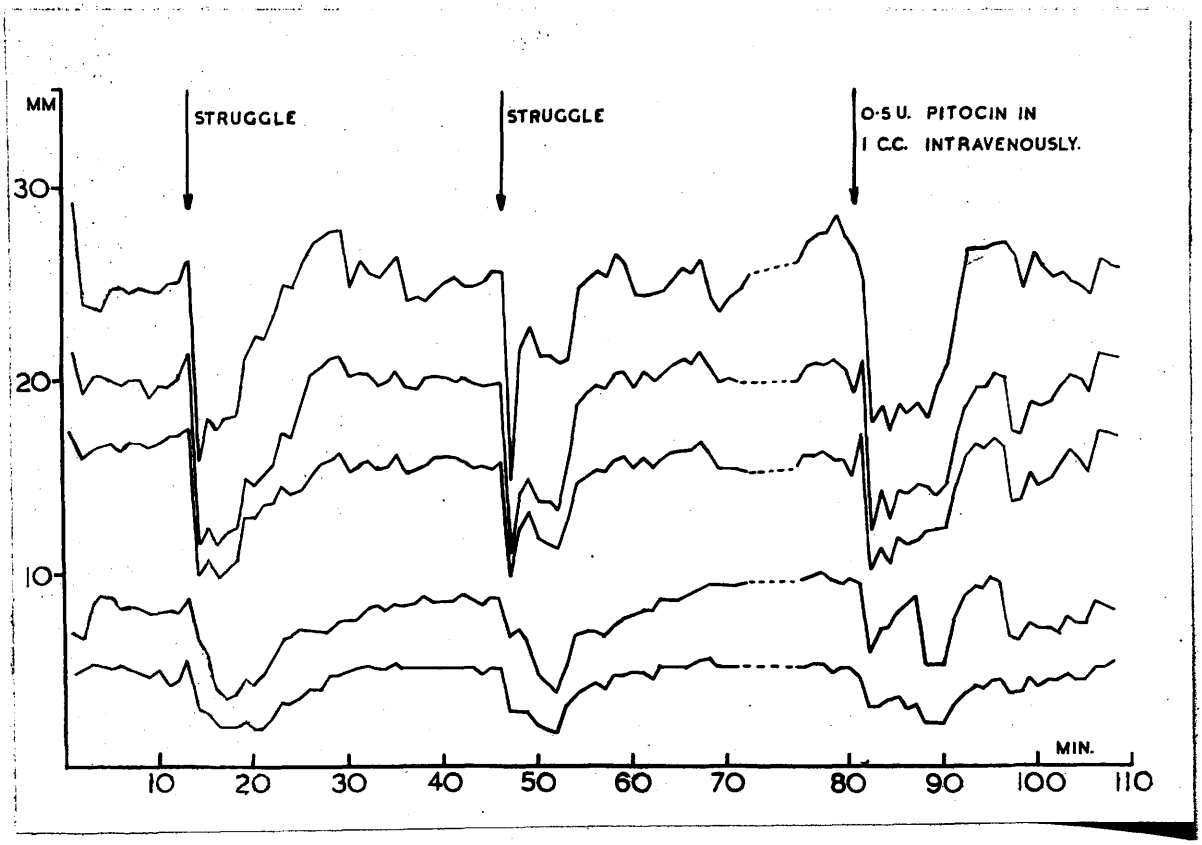

Fig. 5a. Rabbit X 5. Stitches inserted and ovaries removed on 14 April, 1938. From 16 May to 22 May, 1938 0·01 mg. oestrone in oil daily. From 23 to 26 May 0·25 mg. progesterone daily. Experiment 27 May, 1938.

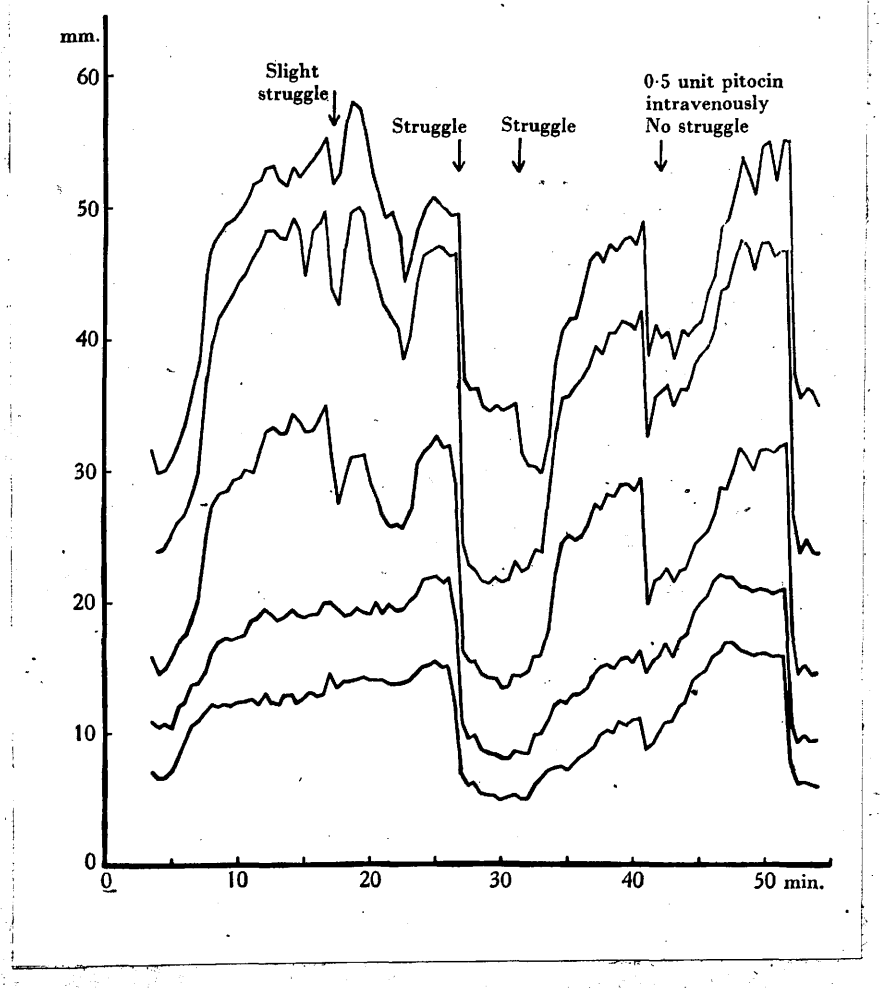

Fig. 5b. Rabbit X 6. Spayed and injected with oestrone and progesterone.

are not quite sharp in every case but in the vast majority the shadows are well defined in spite of the relatively long exposure time of two seconds. This finding effectively disposes of any suggestion that respiration plays a part in moving the stitches as the rabbit breathes about one hundred times per minute. The uterus and intestine were never adherent but presumably the uterus might be pushed about by active coils of intestine. In the case of rabbit X 3 there was a very great difference between the tracing obtained shortly after spaying and that obtained a week later after oestrin treatment. (Fig.3) The alteration in the character of the graph is almost certainly due to alterations in the behaviour of the uterus itself as the intestine is not affected by oestrin. Again, the speed of the movements, especially in X 4, X 5 and X 6, is very much slower than that of intestinal movements.

The shadows of two stitches may move towards one another by a contraction of the intervening part of the uterus or merely by an alteration in the angle formed by the line passing through the stitches and the horizontal, i.e. foreshortening. Consideration of the rabbit's uterus lying, as it does, on the abdominal wall and pressed down on it by the weight of the viscera would suggest that foreshortening, though possible, is not likely to be important. Further in any big contraction it will be seen that, almost without exception, all the interstitch distances decrease

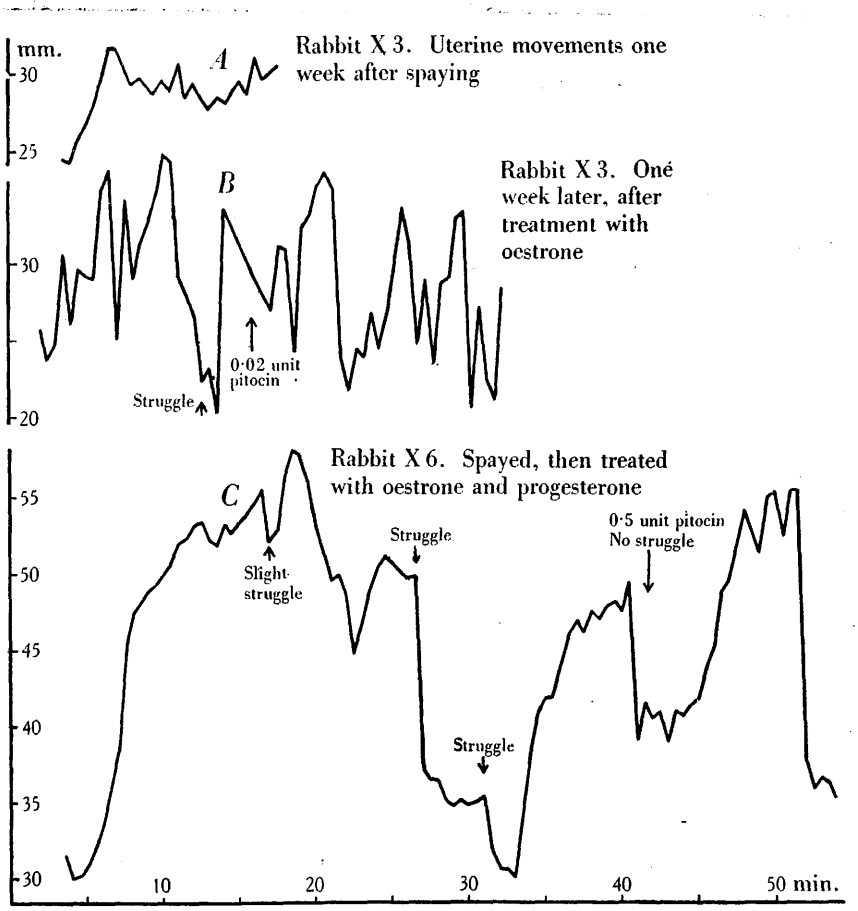

Fig. 5c. Diagram showing the variations in length of the unloaded uterus. A separate length scale in mm. is given on the ordinate opposite each curve. The time scale in minutes on the abscissa applies to all three curves.

simultaneously; it seems unlikely that the whole of the uterus would so regularly change its inclination to the horizontal simultaneously.

Since the rabbit has a very large and lax mesometrium, which permits very great mobility, the experiment was repeated on the cat which has a very short mesometrium. The uterus in this species is held close to the upper, i.e. dorsal, part of the abdominal cavity.

Cat X 2 was spayed and stitches were inserted along the left mesometrium. About two months later it was given 0.05 mg. of oestrone in oil twice a day for ten days. At the end of this period it was decerebrated under ether and the anaesthetic allowed to blow off. The decerebration was performed because it was not thought likely that an unanaesthetised cat would lie quietly for an hour like the more docile rabbit. When marked decerebrate rigidity appeared photographs were taken. The graph (Fig. 6) shows two spontaneous contractions about 28 minutes apart. Immediately after an injection of 0.5 unit of pitocin a large uterine contraction occurred in which the total length of the uterus was reduced to nearly one half of its maximum relaxed length. This contraction was more sustained than the spontaneous contractions. Immediately after the X-ray photographs were completed the uterine movements were recorded by means of the boat shaped cannula described in Chapter Six. The uterus now showed much greater

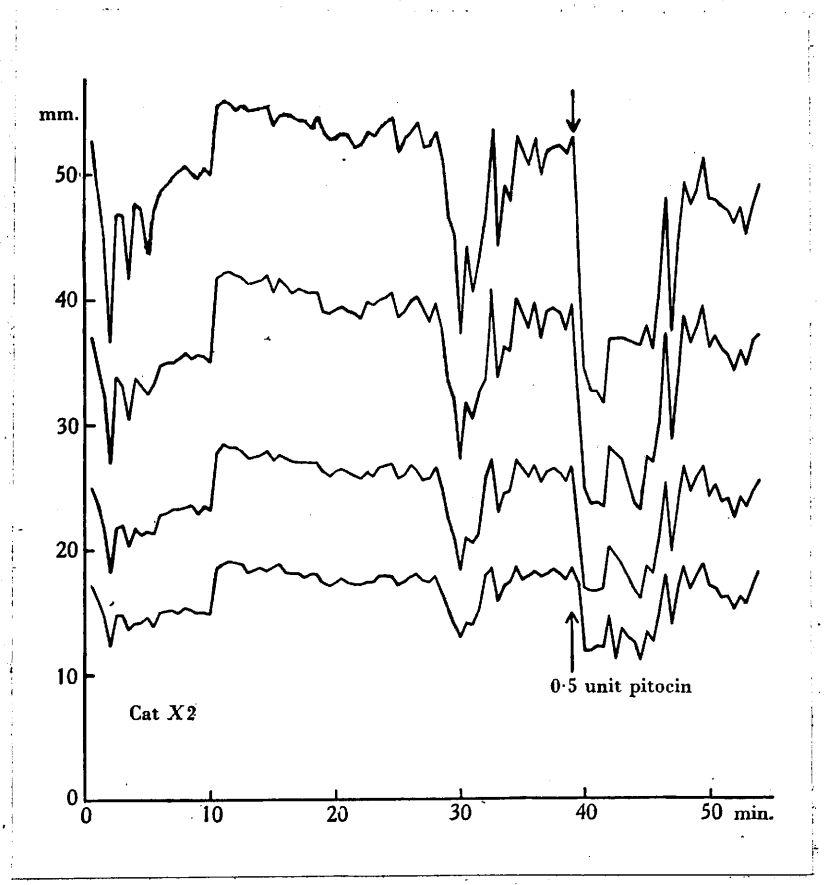

Fig. 6. Cat X 2. Injected with oestrone.

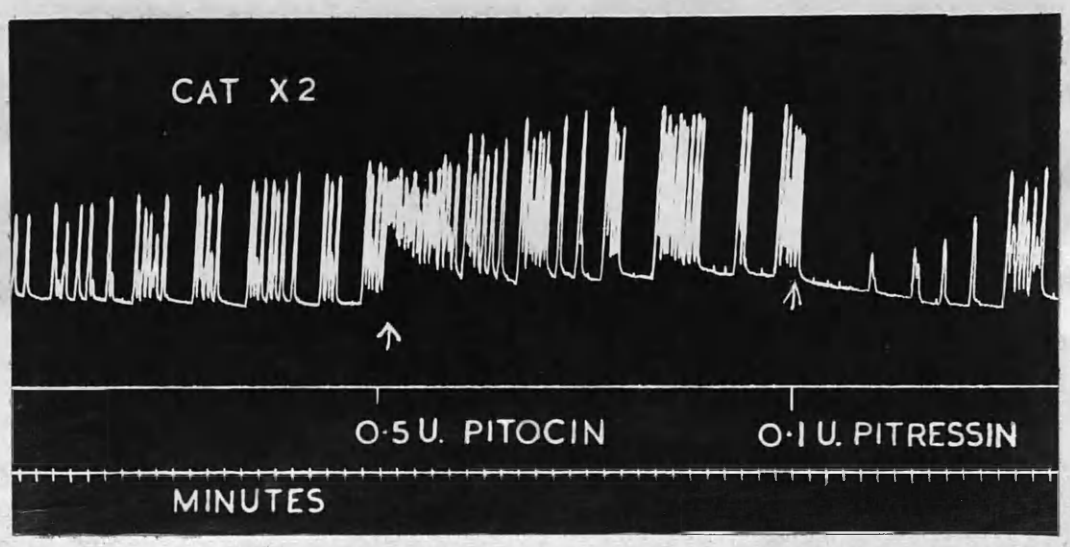

Fig. 7. Cat X 2. In this case the movements were recorded by a lever tied to the uterus and writing on a smoked drum. An upstroke denotes a uterine contraction.

spontaneous activity (Fig. 7); the X-ray method showed complete quiescence for about 17 minutes between the two spontaneous contractions while the maximum interval with the older method is two minutes; the duration of the oxytocic action of pitocin is, however, of the same order. It is interesting to note that, while the reactions to oxytocin and vasopressin are as described by Robson & Schild (1938), the type of spontaneous activity is different. This may be due to the anaesthetic used by them or to the decerebration in the present case.

The finding that the uterine movements can be recorded by the present method even when the mesometrium is relatively tight, makes it even more certain that the movements found in the rabbit are genuine uterine movements on which the tightness or laxness of the mesometrium has little effect.

It would be most interesting to have records of the primate uterus in the unloaded state, because it is in this case that there has been most discussion of the effect of distension. Experiments on two Macacus rhesus monkeys were undertaken, but the graphs obtained were practically straight lines and are not reproduced here. The actual amount of contraction occurring in such a small fibrous uterus (about one inch long) is very small, and may be readily masked by the movements of the uterus as a whole in the pelvis. The X-ray method gives practically no magnification and therefore cannot be expected to give even in

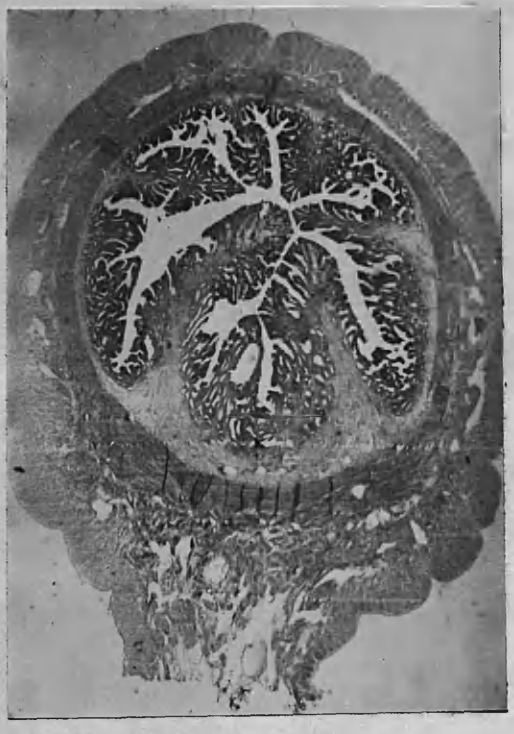

Fig. 8. Sections of uteri of rabbits. Magnification 10 times, except in the last section where it is 11 times. Top left, rabbit X 3 after spaying; top right, the same after injection with oestrone. Bottom left, rabbit X 4 after injection with pituitary powder. Bottom right, rabbit X 5 after treatment with oestrone and progesterone.

large monkeys a good record of uterine movements.

Conclusions

There is no doubt now that the behaviour of the uterus in vitro is a most unreliable guide to its behaviour in vivo. The present work raises doubts as to whether all the work done on uterine movements in vivo can be accepted as it stands.

The most interesting finding in the present experiments is the large amount of spontaneous activity shown by the pseudo-pregnant rabbit uterus. In no other animal has complete quiescence been produced by oestrone and progesterone when the movements have been recorded by ordinary kymographic methods. If the present work is accepted - and there seems to be no great difficulty in doing so - then an anomaly is removed.

It will be obvious that this X-ray method can be used only as a check on the more usual methods of determining uterine behaviour; it is very expensive and the making of measurements and graphs extremely laborious. If, therefore, the findings of the present X-ray method agree with those of previous workers with other methods, then we must assume that the description already available is actually that of the behaviour in the normal animal. This appears to be the attitude to adopt to the experiments on rabbits X 2 and X 3. It seems that the rhythmic contractions of the oestrous uterus are not excited by the weight of the recording lever, but the possibility that the spontaneous

movements may be exaggerated by tension is not excluded, nor is any light thrown on the functional significance of these movements in the non-gravid uterus.

It is quite obvious from this work that Starling's law of the heart is quite inapplicable to the pseudopregnant rabbit uterus. With zero distension, as in the present work, there is considerable movement; with a small amount of distension (Reynolds & Friedman, 1930) there is no movement at all; with greater distension there is considerable activity (Allen & Reynolds, 1935). Recently Reynolds (Reynolds, 1939) has laid much stress on the factor of intra-uterine tension. It will be necessary to reconsider theories of the causation of parturition based on intrauterine tension in the light of the present work. The matter will be discussed again in the concluding chapter.

Summary

A method of obtaining records of the movements of the uterus by taking serial X-ray photographs of silver wire stitches placed in the mesometrium along the uterine border is described. Graphs can be constructed to show movements of the uterus in the absence of any tension or distension, and in the unanaesthetised animal.

The behaviour of the rabbit uterus as a result of spaying and after oestrin treatment is very like that previously described by ordinary kymographic methods. The uterus of a pseudopregnant

rabbit shows long periods of quiescence with intervening large contractions. The uteri of rabbits treated with oestrone and progesterone show very similar movements. These uteri have been previously described as quiescent.

The behaviour of the uterus of a cat treated with oestrone is recorded. It shows rather less activity in the unloaded state as compared with the loaded.

The method is not applicable to the uterus of Macacus rhesus.

The possible causes of the variations shown on the graphs are discussed; it seems certain that they are true records of uterine movements.

The expenses of this work were defrayed out of grants from the Medical Research Council and the Rankin Medical Research Fund of the University of Glasgow.

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Chapter Eleven

THE ASSAY OF OESTRONE IN THE GUINEA PIG

From the Institute of Physiology,
University of Glasgow, 1937-1940.

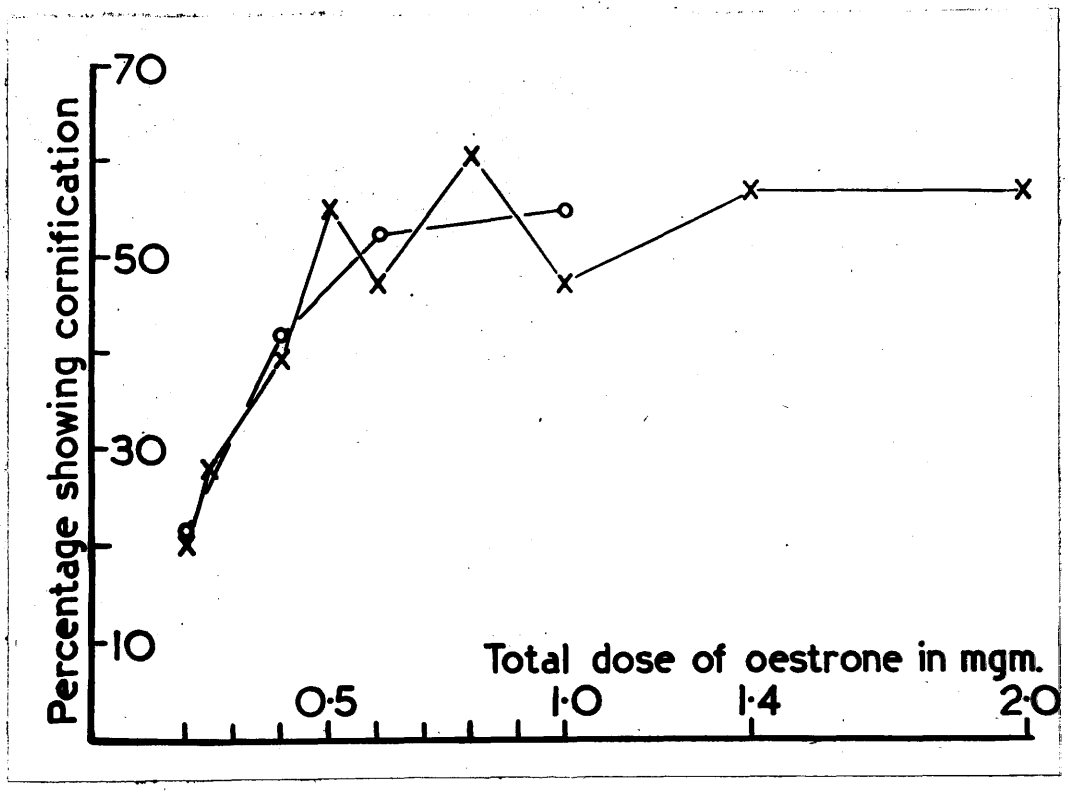
It is now fully appreciated that very great species differences exist in the realm of reproductive physiology and that argument by analogy is a very risky procedure. Hain & Robson (1936), for instance, have shown that there is a greater difference between the rat and mouse units of oestrone, however administered, than can be accounted for on the simple basis of difference of weight. They investigated in great detail the effect of oestrone in oil given in four equal doses over thirty six hours. This chapter describes the results of giving oestrone in this way to the guinea pig. This information was necessary for another reason. The dose of oestrone given to the guinea pigs in Chapter Six was decided on rather arbitrarily and was not based on the authority of experiment. It was important to know if the doses used were within the physiological range because the absence of an effect if the dosage were too small would be a finding of little value, whereas the results of excessive dosage would be only of pharmacological interest.

Method and Results

Smooth haired guineapigs obtained from several dealers were used. They were ovariectomised at about 250 g. A stock of about twenty animals was maintained during the period of the experiment, about three years, by adding new animals as required to replace casualties. The animals gained in weight during the experiment. No injections were given until a month after the removal of the ovaries, or until a month had elapsed since a previous course of injections. The oestrone (British Drug Houses, Ltd., M.P. not lower than 255°C.) was dissolved in alcohol and added to the necessary volume of sesame oil, the alcohol then being blown off; it was injected subcutaneously in four equal doses on the mornings and evenings of two successive days. Vaginal smears were made by means of a loop from the fourth to the ninth day after the first injection; they were taken at intervals of approximately four hours from 8 a.m. to 10 p.m. (since black-out conditions, the last smear has been made at 5 p.m.) The smears were fixed in a mixture of alcohol and ether and stained with methylene blue and eosin; in this way the cornified cells were stained a clear pink whereas all the other cells were blue. In agreement with Hain & Robson a smear was considered positive, or fully cornified, when it contained more than 95% of fully cornified pink staining cells. The presence of leucocytes, but

not of red cells, made a smear negative. Each point on the graph represents the percentage of a group of forty animals giving a positive reaction. Well over ten thousand smears were made and read in the course of the work.

At first a solution containing 0.05 mg. of oestrone per 1 c.c. of sesame oil was used; the results are indicated by the circles on the graph. It can be seen that on increasing the total dose from 0.6 to 1 mg. the number of animals showing full cornification did not increase. It was then supposed that the relatively large volume of oil might be exercising some inhibitory or toxic effect. Accordingly a more concentrated solution containing 0.25 mg. of oestrone per c.c. was tried; the results in this case are indicated by crosses on the graph. This second curve practically coincides with the first; the differences are much smaller than the standard error, which is about 11 p.c. for 40 observations at 50 p.c. In spite of increasing the dosage up to 2 mg. no further increase in the incidence of full cornification was obtained. The smallest dose producing a positive result in 50 p.c. of the animals was about 0.5 mg. in both series. The time of occurrence of full cornification was very variable (from the fifth to the ninth day), but the greatest number of fully cornified smears was obtained on the eighth day after injections began. Initial experiments, in which smearing commenced as soon as the vagina opened, showed that fully cornified smears never occurred



Graph showing the relation between the total dose of oestrone in oil administered to spayed guinea pigs and the percentage of animals showing full vaginal cornification. Each 0 or X represents a group of 40 animals.

0 —— 0 each c.c. of oil contained 0·05 mg. oestrone.

X —— X each c.c. of oil contained 0·25 mg. oestrone.

on the third day, and only very exceptionally on the fourth day, after injections began.

Discussion

Because of the shape of the curve relating dosage to response, and of the variability of the time of response, the guinea pig cannot be considered a suitable animal for the assay of oestrone. Under exactly the same experimental conditions and using the same criteria Hain and Robson found that the dose producing cornification in 50% of animals (unit) was 0.0033 mg. for the rat, and 0.00009 mg. for the mouse. The guinea pig unit, 0.5 mg., is thus about 150 times the rat unit and about 5,500 times the mouse unit. The guinea pigs weighed from 400 to 600 gms. and were thus only two or three times the weight of the rats used by Hain and Robson. The discrepancy between these species cannot, therefore, be explained simply on the basis of difference of body weight.

Dempsey, Hertz & Young (1936) showed that sexual receptivity could not be produced at all regularly in the spayed guinea pig with oestrin only; they say that their results are "difficult to reconcile with the view that oestrin alone is responsible for the production of heat". Oestrus, defined as sexual receptivity, has been produced much more certainly by giving oestrin followed by progesterone. Unfortunately these workers give no record of the vaginal smear picture produced by

this treatment - but it would almost certainly be dioestrous. Using somewhat larger doses than those used by Dempsey et al., it was seen in Chapter Six that a prooestrous smear produced by oestrin alone was replaced by a dioestrous smear when progesterone was given in addition. But there is no doubt that there is a stage in the normal oestrous cycle of the guinea pig when a large proportion of the cells in the vaginal smear are cornified (Stockard & Papanicolaou, 1917; Bacsich & Wyburn, 1940). The last two workers in a personal communication give it as their opinion that the maximum proportion of cornified cells found in a normally occurring oestrus would be about 90 p.c. but that many animals would show a smaller number. Further, leucocytes may persist through all stages of the cycle, although this is not common (Young, 1937). It would appear that our criteria of full cornification - at least 95 p.c. cornified cells with no leucocytes - are artificial and exacting; but the fact remains that it is possible with adequate dosage of oestrin to reach this high standard in 100 p.c. of rats and mice.

It is difficult to find any explanation of this failure to produce full vaginal cornification in more than about 55 p.c. of guinea pigs. The possibility that the oily vehicle was toxic seems to have been ruled out. The shape of the curve does not suggest that even higher dosage would have been effective; the rat and the mouse curves show no indication whatever of a falling

off at 50 p.c. It is unlikely that the anterior pituitary gland could play any part since its hormones do not appear to act directly on the vagina. It may be that the spacing of the injections used in this work is not the optimum for the guinea pig, but the exploration of this question would have turned the work away from its main purpose, namely, to make a direct comparison between the guinea pig and the rat and the mouse. If regeneration of ovarian material had occurred (see Parkes, Fielding & Brambell, 1927) this might account for the falling off after 50 p.c., especially as the higher doses were given at the end of the experiment. When the ovary was removed the whole of the uterine tube and a portion of the uterine horn was also taken away; regeneration in these circumstances is unlikely. Further, animals which did not react to the highest dosage of oestrone were examined histologically for evidence of ovarian regeneration with negative results.

Summary

Spayed guinea pigs were injected with oestrone in oil (four injections in thirty six hours). 0.5 mg. oestrone produced full vaginal cornification in 50 p.c. of the animals; this is many times the dosage required in rats and mice, when allowance is made for differences in body weight. Increasing the total dose of oestrone to 2.0 mg. produced very little increase in the number showing full cornification, but no explanation of this result can be offered.

Dr.A.McL. Watson gave very valuable advice on the staining of the smears and the examination for ovarian regeneration. The expenses were defrayed by grants from the Medical Research Council and the Rankin Medical Research Fund of the University of Glasgow.

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Addendum to Chapter Eleven

An interesting sequel to this piece of work will be described very shortly as it has no direct bearing on the subject of reproduction but is rather of pharmacological interest.

When the final smears of the experiment had been read and graphed it was necessary, to comply with the conditions of the licence for experimenting on animals, to kill off the remaining animals. (Some had already been killed for the investigation of ovarian regeneration as already described). This was actually done several months after the last injection when it was certain that it would not be necessary to prolong the experiment further. It was then found that some of the animals had irregularly shaped kidneys resembling to a slight extent the granular contracted kidneys of human pathology. Corresponding to the depressed parts wedge shaped areas of collapsed but still living renal tissue were seen on microscopic examination. The glomeruli were shrunken and contained only a few red cells whereas adjacent healthy glomeruli were large and well filled with blood. The tubules corresponding to the damaged glomeruli were collapsed with no lumen and the cells were small. Further down in the medulla large dilated tubules containing granular material were found. There was a slight overgrowth of fibrous tissue. The greater part, say two thirds, of the kidney appeared to be healthy. Prof.Shaw Dunn, who very kindly looked at the specimens, was of the opinion that this

was a naturally occurring condition and not likely to be related to the previous treatment.

It was felt, however, that the point was sufficiently important to require investigation. Since oily injections containing oestrone and other materials are given frequently to women it would be rather unfortunate if kidney damage at a later date were to follow.

Three groups of young female guinea pigs were taken; the first group of seven animals received 2 c.c. of natural sesame oil twice weekly; the second group of seven animals received the same amount of sesame oil which had been autoclaved; the third group of six animals acted as controls and received no treatment. It was not thought likely that oestrone itself would cause kidney damage since large quantities of oestrone or very nearly allied substances are excreted by the kidney during pregnancy without causing any damage to that organ. The sesame oil or perhaps its breakdown products, was more likely to be the cause of the trouble and for this reason autoclaved oil might be expected to be more toxic than the natural oil. Autoclaving seemed to be the best method of increasing the break down products without introducing any new material to complicate the issue.

The injections were kept up over a period of seven months. Not all the animals lived for this time, some died of pneumonia due to exposure before this because of the draughty condition of the animal room as the result of the "blitz" of

March, 1941 (the experiment lasted from the 3rd.of February to the 11th. of September, 1941). The injected animals did not live so long as the controls.

After examination of all the material it was found that there was no difference between the three groups in respect of kidney damage which was in all cases very slight indeed; there was no real case of wedge shaped necrosis. The conclusion seems to be, as far as these tests go, that there is no danger of damage to the kidneys from large injections of sesame oil. This is certainly reassuring.

The wedge shaped fibrosis seems to be an accompaniment of old age in guinea pigs arising "spontaneously". The guinea pig does not seem to be the only animal which suffers in this way as very similar lesions have recently been found in the kidneys of rabbits which had not been experimented upon.

Chapter Twelve

THE BEHAVIOUR OF THE PREGNANT UTERUS OF THE GUNIEA PIG

From the Institute of Physiology, University of Glasgow, 1939-40.

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 - (see Chapter One) 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 Chapters Two and Four) 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, 1933b), in the rabbit (Robson, 1933a), 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 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 (see Chapter One).

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 days on which the vaginae were open were recorded. The vaginae were open for three days on the average, with a range of one to seven. 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 three weeks onwards and to estimate roughly its duration. Ishii (1929) 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. per kg. subcutaneously) repeated as required. A large number of anaesthetic deaths occurred in early pregnancy, but animals more than one month pregnant rarely gave any trouble. The external jugular vein was cannulated; the abdomen was opened and a boat-shaped cannula (described in Chapter Six) 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. No photoelectric amplification was required. 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 standardisation 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 in 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's solution.

Results

Typical records from the living animal are shown in Figs. 1 and 2, in 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

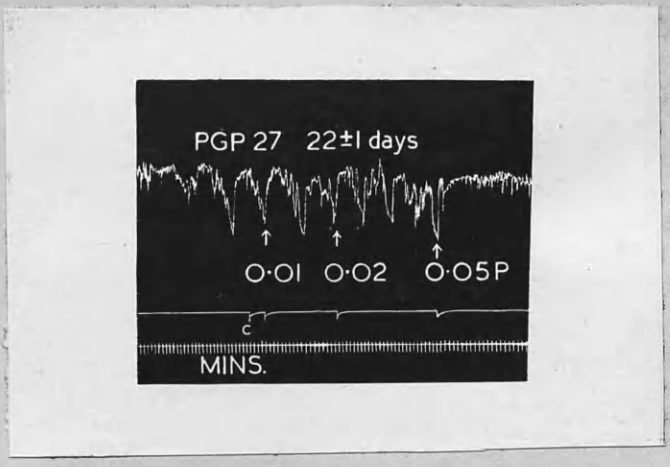

Fig. 1. PGP 27, 22±1 days pregnant. Reaction to 0·05 unit of pitocin intravenously; no reaction to smaller doses. At C chloralose given subcutaneously.

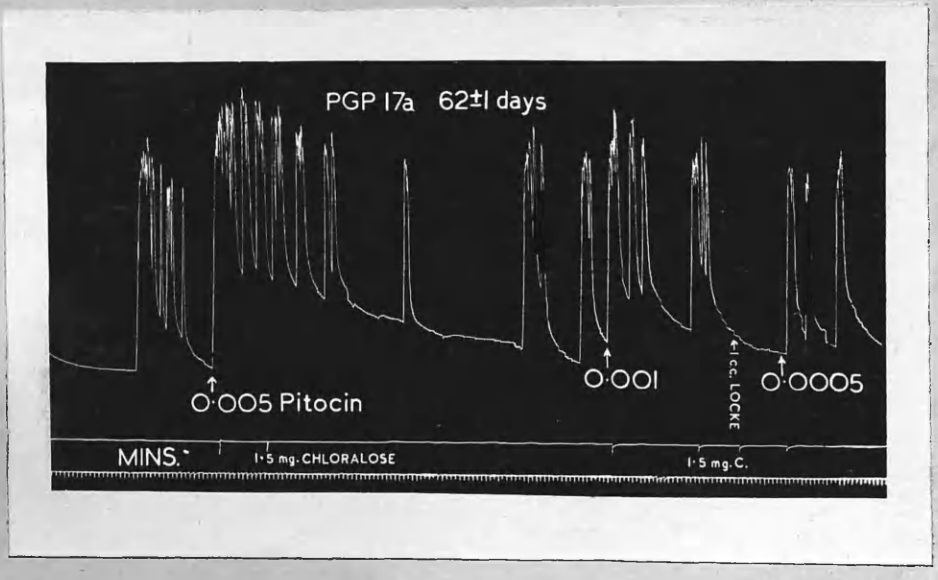

Fig. 2. PGP 17a, 62±1 days pregnant. Reaction to 0·001 unit of pitocin intravenously; smaller doses of pitocin and control dose of plain Locke solution without effect.

is not by any means regular and it is not easy to distinguish tracings (except by amplitude) made early in pregnancy 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).

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. 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 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

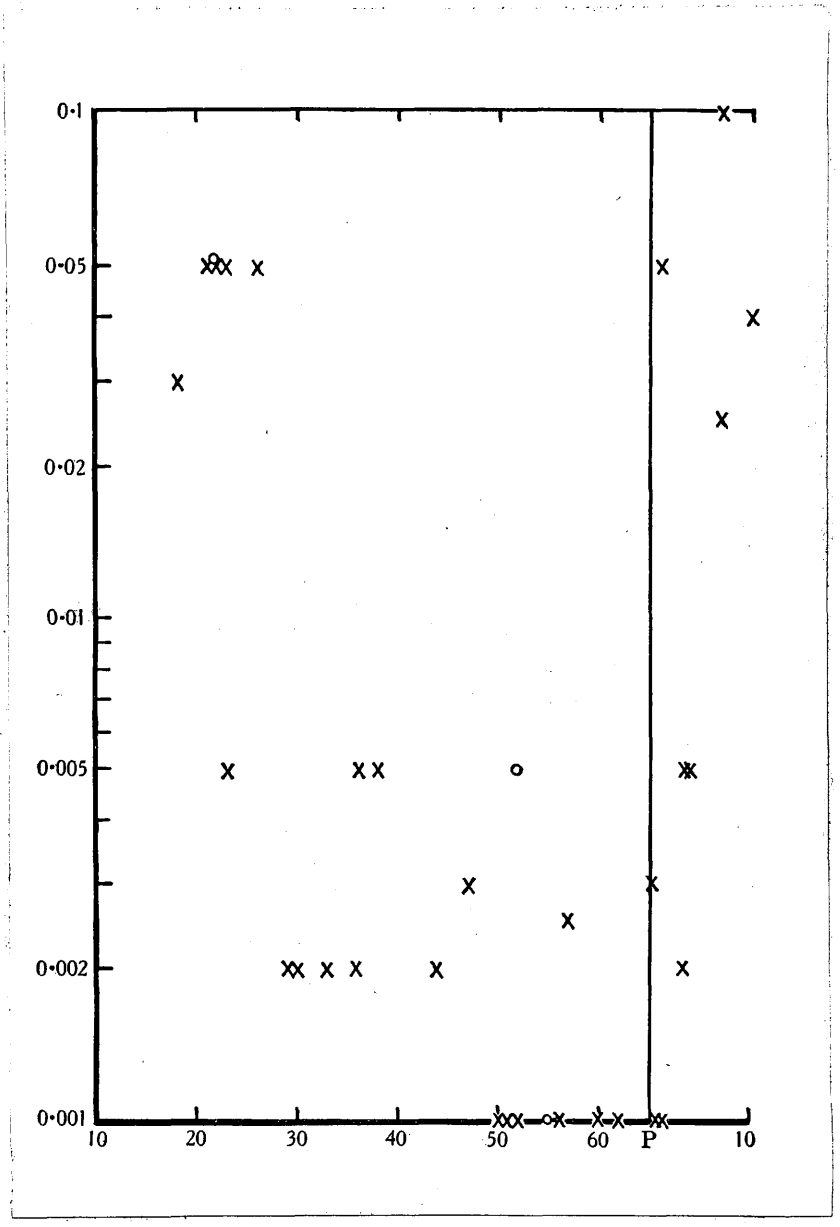

Fig. 3. The threshold dose of oxytocin on the ordinate at various stages of pregnancy on the abscissa. x denotes that the duration of pregnancy was timed from the opening of the vagina; o means that the duration of pregnancy was estimated from the graph of Fig. 4.

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 $y = -0.417 + 0.00407x^{1.924}$, where y is the length of the foetus in cm. and x the duration of the pregnancy in days. I am indebted to Dr.Hague of the Electrical Engineering Department for indicating to me the method of deriving the equation. 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 o in Fig. 3; an x in Fig.3 indicates that the duration of pregnancy is reckoned from the

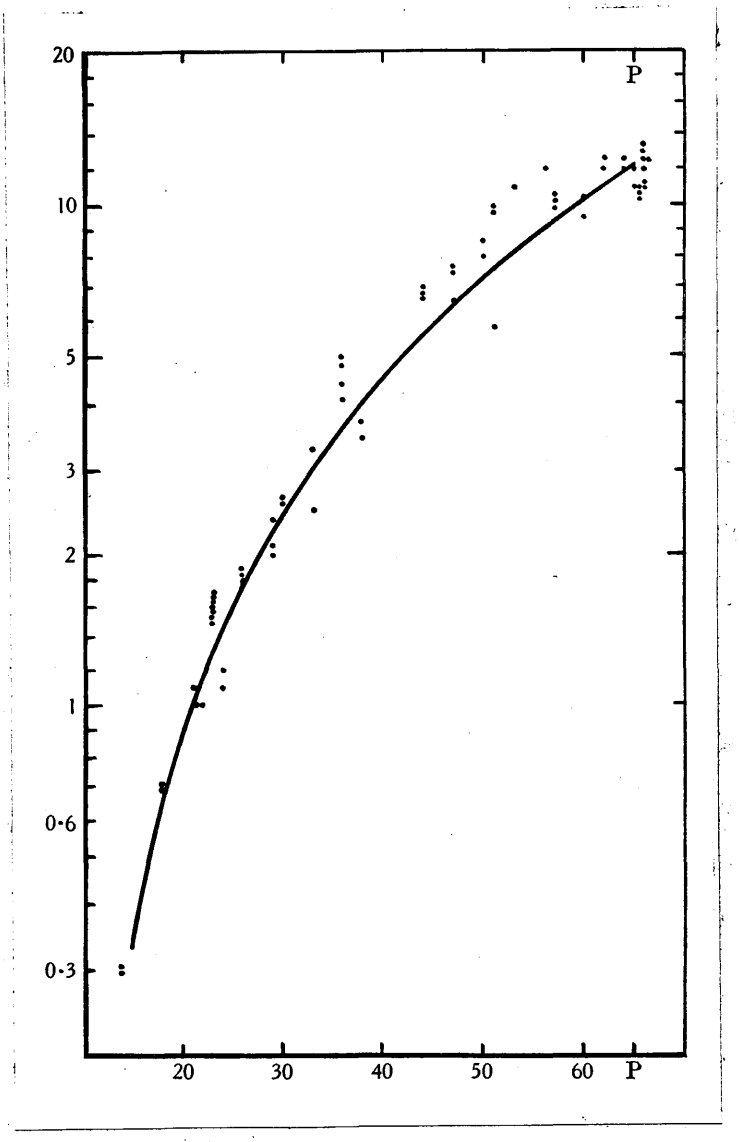

Fig. 4. This graph shows the nose to rump lengths in cm. - plotted on a logarithmic ordinate - of all foetuses whose age was known. The duration of pregnancy in days is given on the abscissa. The line has been drawn to the equation

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

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

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 sensitisation of the uterus to oxytocin, but since the uterus shows a high reactivity to oxytocin during the last fortnight of pregnancy this sensitisation 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 (see Chapter Seven). 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 (see Chapters One, Two and Four).

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 also the rate of flow increase as pregnancy advances; but at the same time the mass of the uterus increases. 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 and 1911) the corpus luteum of the guinea pig is fully formed about five days after ovulation and remains till the fortieth 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 Chapter Six 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 will be described in Chapter Thirteen. A reinvestigation of the earlier work is necessary 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 Chapter Six, 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 coordinates 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. It was shown in Chapter Six, however, 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, (Chapter Six) has been confirmed. The weights of the pregnant pigs 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 co-efficient is +0.24 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 (Chapter Ten) concerning the reliability of observations made by levers producing tension on the uterus, and in spite of the well-recognised 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.

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Chapter Thirteen

A REINVESTIGATION OF THE ACTION OF OVARIAN HORMONES ON THE GUINEA PIG UTERUS WITH SPECIAL REFERENCE TO THE HORMONE OF THE CORPUS LUTEUM

From the Institute of Physiology, University of Glasgow, 1941.

It was shown in Chapter Six that progesterone had no effect on the spontaneous activity or the reactivity to oxytocin of the non-pregnant uterus of the guinea pig when the observations were made in the living animal. In Chapter Twelve experiments on the activity and reactivity of the guinea-pig uterus in the living animal during pregnancy were described; it was found that in early pregnancy the uterus was comparatively unreactive, and that it became more reactive to oxytocin as the time of parturition approached. The corpus luteum of the guinea pig is, according to histological criteria, active during the unreactive uterine phase and is declining as the uterine reactivity rises. This alteration in the reactivity may only be associated with changes in the corpus luteum and may not be produced by them; before making a plunge into speculation and suggesting that we must search for still more hormones it would be advisable to look more closely into the early experiments described in Chapter Six. These experiments were carried out before progesterone was readily available and it may quite well have been that the dosage of the hormone administered at that time was inadequate to bring about the changes in

reactivity found in early pregnancy.

This chapter will describe the reinvestigation of the early work under better experimental conditions and an extension of the observations to see if the naturally occurring corpora lutea have any effect on uterine behaviour.

Method

The animals can be divided into two groups - one spayed and the other normal intact animals. The latter were used either at oestrus when ripe follicles are present in the ovary or about one week after oestrus when corpora lutea are found. It was found impossible to produce corpora lutea by injections of gonadotrophic hormones; this is in sharp contrast to the rabbit where a small injection of gonadotrophic hormone can produce massive luteinisation of the ovary of the oestrous animal. Dried mare anterior pituitary powder of known potency which had been tested on rabbits, and also various hormone preparations of serum and of urine of pregnancy very kindly supplied by Dr.Macbeth of Organon Ltd. were all without effect. In the experiments where the uterus was under the influence of the animal's own corpora lutea it is doubtful whether the amount of luteal tissue was as great as would be present in the ovaries during a pregnancy; unfortunately there seems to be no way out of this difficulty. The spayed group were treated with oestrone and progesterone exactly as in the earlier experiments except that the range of dosage was increased. The

experiments described in Chapter Eleven suggested that a slightly higher dosage of oestrone would be more physiological, and the considerations mentioned in the introduction to this chapter led to the use of quite large doses of progesterone. The details are given in Table I. The spayed animals were treated with oestrone in oil for seven days and on the seventh day were given also the first dose of progesterone, this dose was repeated on the eighth and ninth days, the observation of the uterine movements being made on the tenth day; in the controls where no progesterone was given there was an interval of two days after the last injection of oestrone, the uterine movements being recorded as before on the tenth day. The progesterone was kindly supplied by Messrs. Schering as Proluton.

The movements of the uterus were recorded under ether anaesthesia using the boat shaped cannula; no photoelectric magnification was necessary and the movements were recorded directly on smoked paper by a lever connected by a thread to the centre of the uterus. In the early experiments (Chapter Six) the jugular vein was merely exposed so that a fine needle could be inserted into it for the injection but in the present series the jugular vein was cannulated so that many more injections could be made with a minimum of trouble. A special non-clotting cannula was devised and is illustrated in Fig.1. This is perhaps a minor detail but it has proved its value many times over. The cannula is filled

with Locke's solution and is tied into the vein in the usual way. The drug to be administered is made up in Locke solution and is injected through the rubber stopper of the cannula using a fine needle pushed well down the cannula; the cannula is washed out using another syringe containing plain Locke solution; in this case the needle is pushed only a short distance through the rubber stopper. When a needle is withdrawn from this cannula there is practically no alteration in volume and no blood is sucked back and therefore no clotting can take place. The tiny rubber stoppers are very easily produced by means of a leather punch which, owing to the pressure required in cutting out of sheet rubber, makes a waisted stopper which cannot readily be pulled out of the glass cannula. It is hoped that by an extension of this principle it will shortly be possible to produce non-clotting arterial cannulae for recording arterial blood pressure without the use of anticoagulants; this has always been an unfulfilled ambition of physiologists who are concerned with investigations of the circulation. Further, there is no reason why this kind of cannula should not be used clinically where there is some indication for repeated intravenous injections; the cannula and the stopper can readily be sterilised - but the stopper cannot easily be inserted into the cannula unless both parts are dry.

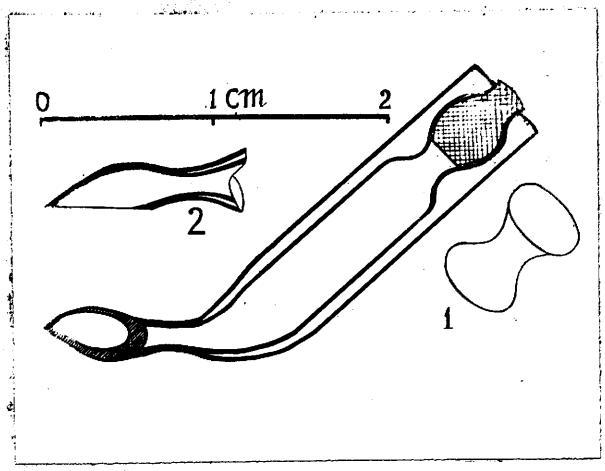

Fig. 1. Non-clotting venous cannula. The large sketch shows the shape of the cannula which is made of glass. The shape of the rubber stopper obtained by means of the leather punch is shown at 1. It is also shown cross-shaded in the large diagram. The cannula may be of any size; the scale of centimetres indicates the size used in the case of the guinea pig. A side view of the point of the cannula is given at 2.

TABLE I.

Animal no. CB	Weight g.	Spayed	Oestrone mg. per day	Progesterone, mg. per day	Threshold dose of oxytocin o.u.	Wave duration min.	Wave height cm.
16	330	Yes	-	-	0.01	1	0.3
17	298	Yes	-	-	0.003	1	0.2
18	340	Yes	-	-	0.01	3	0.2
9	377	Yes	0.02 for 7 dys.	-	0.003	5	11.0
30	490	Yes	as CB 9.	-	0.005	8	5.5
8	350	Yes	as CB 9.	-	0.006	4	7.0
19	410	Yes	as CB 9.	-	0.008	8	5.0
35	340	Yes	as CB 9.	-	0.03	4	2.0
14	407	Yes	as CB 9.	-	0.1	6	6.0
24	439	Yes	0.05 for 7 dys.	-	0.003	10	5.0
20	355	Yes	as CB 24	-	0.005	9	10.0
5	360	Yes	as CB 24	-	0.01	10	7.0
31	380	Yes	as CB 24	-	0.03	4.8	1.8
10	277	Yes	0.02 for 7 days	2.0 for 3 days	0.005	-	-
21	340	Yes	as CB 10	as CB 10	0.05	-	-
11	282	Yes	as CB 10	as CB 10	0.09	1.3	11.0
32	527	Yes	0.02 for 7 days	5.0 for 3 days	0.008	1.2	6.5
33	331	Yes	as CB 32	as CB 32	0.05	7	8.0
6	-	Yes	0.05 for 7 days	5.0 for 3 days	0.002	3	1.5
13	431	Yes	as CB 6	as CB 6	0.03	-	-
7	350	Yes	as CB 6	as CB 6	>7.0	2.7	7.0
22	340	Yes	0.05 for 7 days	10.0 for 3 days	0.05	16	7.0
23	315	Yes	as CB 22	as CB 22	0.1	16	10.0
27	356	No	Large follicles	-	0.001	1	1.0
26	375	No	as CB 27	-	0.01	16	8.0
34	457	No	-	Corpora lutea	0.02	6	5.0
25	364	No	-	as CB 34	>2.2	-	-

Results

These confirm to a remarkable degree the results already described in Chapter Six. The three spayed animals showed less activity, i.e. smaller waves of shorter duration, than the oestrin treated group but there is no difference in their reactivity to oxytocin. This agrees with Chapter Six. In the animals treated with oestrone and progesterone the range of threshold doses of oxytocin (i.e. minimum dose required to produce a uterine contraction) overlaps the range of threshold doses in the animals injected with oestrone only. The results can be grouped for purposes of comparison as in Table II. This distribution is not likely to have arisen by chance as it gives a

TABLE II.

Threshold dose of oxytocin o.u.	Oestrin-treated, or follicles in ovaries	Progesterone-treated or corpora lutea in ovaries
Up to 0.01	9	3
Over 0.01 and up to 0.1	3	7
Over 0.1	0	2

χ^2 value of 6.6 (the value of χ^2 for P = 0.05 is 5.991). Omitting the experiments in which no spontaneous activity was found, i.e. four cases among the progestin group, the average duration of a single spontaneous wave in the progestin group is

6.7 minutes as compared with 7.2 minutes in the oestrin group; in the spayed untreated group it is 1.7 minutes. Including the four cases which showed no spontaneous activity the average height of the spontaneous waves at the beginning of the experiment was in the progestin group 5.4 cm., in the oestrin group 5.8 cm., and in the spayed untreated group 0.2 cm.

Discussion

The most striking and most baffling feature of the results is their variability with resultant overlapping between the various groups. When a comparison is made with results obtained in the pregnant guinea pig described in last chapter two points require to be considered:- first the range of reactivity found in the pregnant animal is covered by the range found here, and second higher thresholds are found more often in animals under the influence of progesterone or in the luteal phase of the cycle. In spite of the difficulty in explaining the low reactivity in the early part of pregnancy it does not seem necessary at this stage to invoke the action of any new hormone. In spite of efforts to vary the actual amount of progesterone used and to vary the ratio of progesterone to oestrone it has to be admitted that the conditions under which the uterus becomes less responsive to oxytocin are not yet clearly defined. One might be tempted to hazard a guess that the state of growth or tension of the uterus might have an influence on the results

but in view of the work on the unloaded uterus (Chapter Ten) speculation on this point is best avoided.

Summary

The previous work on the guinea pig uterus is confirmed. In spite of the scatter of the results there is an undoubted tendency for progesterone to reduce the response of the uterus to oxytocin.

The expenses of this work were defrayed out of a grant from the Rankin Medical Research Fund of the University of Glasgow.

Chapter Fourteen

THE BEHAVIOUR OF THE UTERUS OF THE GOLDEN HAMSTER (CRICETUS AURATUS) UNDER THE ACTION OF VARIOUS HORMONES.

From the Institute of Physiology, University of Glasgow, 1940.

These experiments were carried out on golden hamsters kindly given to me by Professor Hindle who was reducing his stock of these animals at the beginning of the war. This afforded an opportunity of testing out the behaviour of yet another species in the search for the fundamental pattern of uterine behaviour. The present report refers only to the non-pregnant uterus; some day it may be possible to investigate the pregnant uterus of the hamster for comparison with the work already done on the pregnant guinea pig.

The golden hamster is remarkable in that its oestrous and gestation periods are exceedingly short, being four and fifteen days respectively (Bruce & Hindle, 1934). In these circumstances it seems right to expect that the action of hormones would occur quickly and after relatively small doses. There is as yet very little information about the "physiological" dosage of hormones in the hamster; unfortunately this takes a long time to acquire (compare the assay of oestrone on the guinea pig in Chapter Eleven). In the work to be described fairly large doses of oestrone and of progesterone were administered (in comparison

with the rat or rabbit dosage) in the hope that if either of these hormones did in fact produce an alteration of uterine activity then certainly sufficient had been given to produce a maximum result.

Methods

The preliminary ovariectomy was performed through a dorsal mid-line incision in the skin which could be moved over to either side for the exposure of the ovaries through the flat muscles of the abdomen; the ovaries were removed with the aid of a red hot electric cautery and no stitches were required in the uterine stump or in the abdominal muscles.

The uterine movements were recorded exactly as in the case of the guinea pig and mouse by means of the boat shaped cannula. The animals were anaesthetised with 1 mg. of chloralose given subcutaneously in about ½ c.c. of warm Locke solution; about one hour after the first injection an additional ½ mg. of chloralose was given. The cannula was 2.8 cm. long, i.e. the size used originally for the mouse. The magnification of the uterine movements by the lever was twenty times; no photoelectric amplification was necessary in spite of the relatively small size of the uterus.

The external jugular vein was cannulated; previous practice with the much larger guinea pig enabled this difficult procedure to be undertaken with a much smaller vessel. It is

interesting to note how one's attitude to veins has altered in the course of this work. At first it was thought that the veins of the guinea pig were too small to cannulate but now even the smallest veins are tackled, if not with complete confidence, at least with hope. Cannulation is a very great help because repeated injections can be given at any chosen moment and thus the threshold dose of oxytocin much more accurately determined than in the earlier experiments.

A vaginal smear was made during the recording of the uterine movements and was stained with eosin and methylene blue as in the case of the guinea pig smears described in Chapter Eleven.

Results

These can be divided into two parts; the first deals with normal animals and the findings are given in Table I.

Table I.

Animal No.	Vaginal smear (read as in the mouse)	Wave height cm.	Wave duration min.	Threshold dose of oxytocin	Average external diam. of uterus mm.
H 3	Prooestrous	0.5	1.0	0.0005	3.0
H 5	Prooestrous	1.1	0.5	0.003	2.0
H 8	Oestrous	1.2	0.5	0.005	1.6
H 9	Oestrous	1.0	0.9	0.005	2.0
H 1	(Early	0.8	1.0	0.001	-
H 4	(postoestrous	1.2	0.9	0.001	1.8
H 6	(Later	1.5	0.9	0.001	1.7
H 7	(postoestrous	0.5	0.3	0.002	1.2

According to Deanesly (1938) it is impossible to trace the stages of the oestrous cycle in the hamster by vaginal smears; she also states that there is no characteristic alteration at any period of the cycle except that at oestrus the uterus is larger. The first two experiments of Table I. the uteri were relatively large and the smears showed large numbers of epithelial cells which Peczenik (personal communication) on the basis of his experiments on mating believes are characteristic of oestrus. It may be then that H3 and H5 are oestrous animals whereas the others are in the interval between successive periods.

The findings of Table I. can be summarised thus: the average wave height was 1.0 cm. with an average duration of 0.8 min. and the average threshold dose of oxytocin was about 0.002 o.u. If the results are considered from the point of view of deviation from these mean values it will be seen that there is no substantial variation throughout the oestrous cycle.

The second part of results deals with the effect of ovarian hormones on the activity of the uterus after ovariectomy. The results are given in Table II.

TABLE II.

Animal No.	Treatment	Wave height cm.	Wave duration min.	Threshold dose of oxytocin	Average external diam. of uterus mm.
H 10)	Spayed one week	0.4	0.8	0.005	1.8
H 11)	before.	1.0	0.7	0.007	1.3
H 12)	Postoestrous smears.	1.0	0.5	0.005	1.4
H 13	Spayed, no treatment for 1 wk., then 25 microg. oestrone in sesame oil per day for 2 days. Smear oestrous.	1.7	0.7	0.01	2.5
H 14	As H 13 but 3 days oestrone. Smear not recorded.	3.0	0.7	0.01	2.5
H 15	Spayed, no treatment for 1 wk., then 25 microg. oestrone in oil for 2 days. Next day 1 mg. progesterone. Final expt. next day.	5.0	0.8	0.002	3.5
H 16	As H 15 but progesterone given on 2 days (1 mg. per day). Smear postoestrous.	9.0	1.2	0.003	3.0
H 17	As H 16. Smear, postoestrous.	5.0	0.5.	0.01	2.4
H 18	As H 15 but three days 1 mg. progesterone per day. Smear not recorded.	4.0	0.5	0.01	1.9

It will be seen that the threshold dose of oxytocin and the duration of the spontaneous waves were not affected by spaying, oestrone or progesterone. Oestrone did, however, increase the height of the waves and when followed by progesterone the increase in wave height was still greater as compared with those produced by the castrate uterus. The size of the waves, as might be expected, was closely correlated with the size of the uterus; the only violent exception to this is H 18 in which the uterus was nearly as small as the spayed uteri but which gave contractions at least four times as great.

Whether this reduction in size is a specific effect of progesterone or an accidental finding due to some variation in the fixing is difficult to say. The smears obtained in this second group were just those that would be expected in the mouse or guinea pig, where the oestrone effect is inhibited by progesterone; but the series is very much too small to venture to criticise Deanesly's findings. There is, fortunately, sufficient evidence in the variation of size of the uteri that the oestrone and progesterone were absorbed.

Pitressin was injected in the course of four experiments (H 5, 6, 9 and 16) and in all produced a slight motor response. The sample used was the usual commercial standard of Parke, Davis & Co. containing about 0.01 oxytocic unit with each pressor unit. All the effects can quite well be explained on the basis of this

incomplete separation and it would therefore appear that vasopressin is without effect on the hamster uterus.

Adrenaline was given in five experiments (H 6, 7, 8, 9, 11) in doses of from 2.5 to 10 mivrogrammes. In all cases a relaxation or temporary inhibition of uterine movements resulted.

No experiments were carried out in vitro since the work described in previous chapters has shown that it is of little value. Representative tracings are given in Figs. 1, 2 and 3.

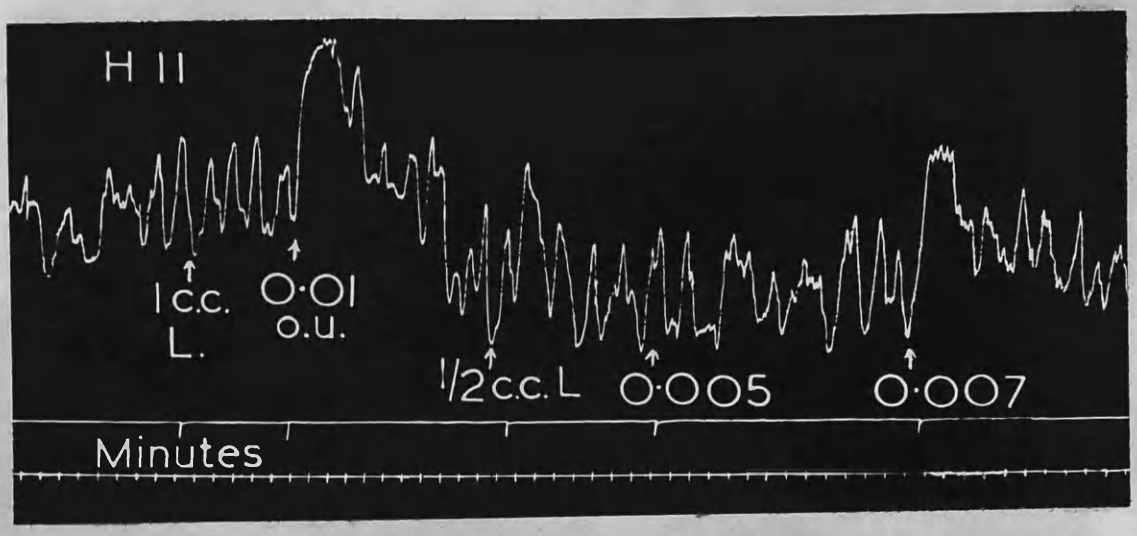

Fig. 1. H 11. Uterine movements (in the living animal) of a hamster spayed one week before the recording of the movements. Motor effect with 0·007 o.u. purified Pitocin.

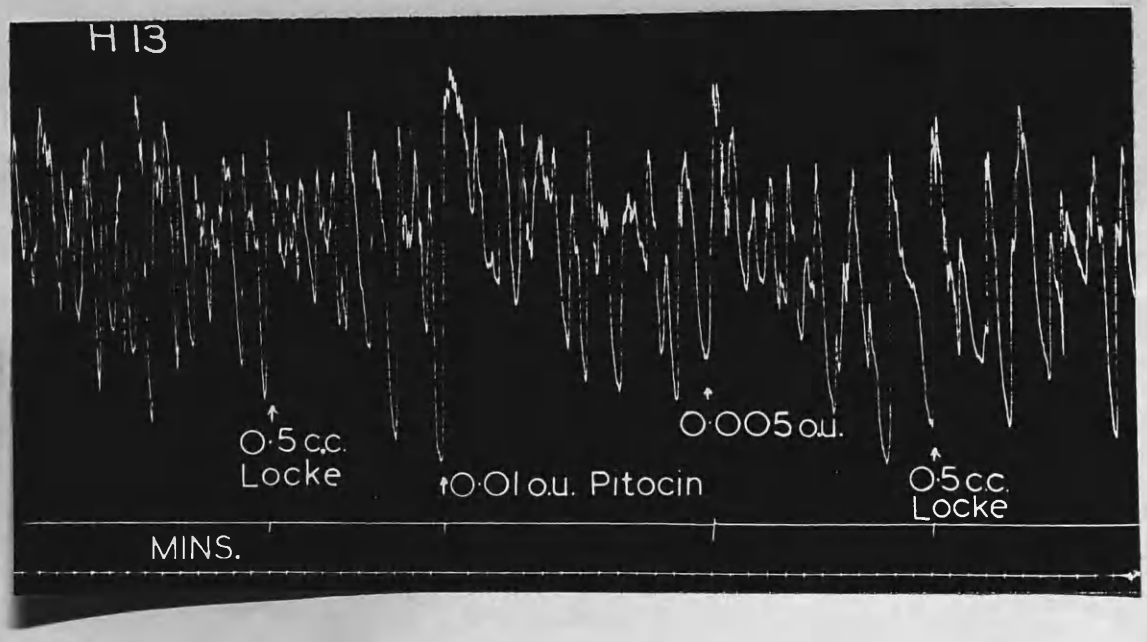

Fig. 2. H 13. Uterine movements in vivo of hamster spayed then treated with oestrone. Motor effect with 0·01 o.u. of Pitocin.

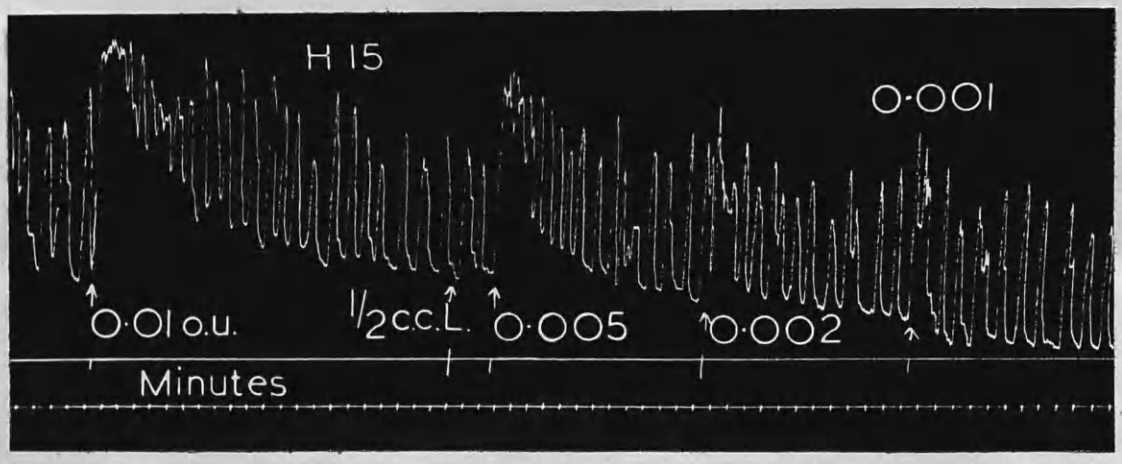

Fig. 3. H 15. Uterine movements of the hamster in vivo. The animal was spayed and then treated with oestrone and progesterone.

Discussion

There is fairly general agreement between the present results, at least as far as spontaneous activity and reactivity to oxytocin are concerned, and those obtained previously with the mouse, guinea pig and monkey uteri. There is a slight resemblance to the rabbit uterus when investigated by the method described in Chapter Ten, in the increase of amplitude of movements after oestrone and still more after oestrone and progesterone; but there is no alteration of the threshold to oxytocin as in the rabbit. (It should be stated that the threshold dosage of oxytocin in the unloaded rabbit uterus is still unknown).

The effect of vasopressin on the uterus of any species seems to be quite unpredictable; but since no place for it has yet been found in the story of parturition the matter may be left on one side for the moment. It would, however, be interesting to know if the posterior lobe can secrete oxytocin and vasopressin separately - experiments to test this would be rather difficult to devise unless some means could be found to sensitise the animal to vasopressin. The difficulty arises from the fact that the blood pressure is so well regulated that only relatively violent drugs (and vasopressin is hardly in this class) produce obvious changes in it. It appears to be rather difficult to separate oxytocin and vasopressin by chemical means and it is natural to enquire if the body succeeds, as it so often does, where the chemist fails.

In general the results produced by treatment of the hamster uterus with ovarian hormones are similar to those found in other animals but there are differences in detail which still defy explanation.

Klein (1938) has shown that - as in the rabbit, mouse, rat and cow - ovariectomy of the hamster always resulted in the termination of pregnancy; also that the pregnancy could be maintained after ovariectomy by injections of 1 microg. oestrone in oil + 0.2 mg. progesterone in oil per day. It is not possible to give any explanation of these findings in terms of the present work on uterine movements since neither presence nor absence of these hormones makes any great difference to reactivity to oxytocin. The tendency for oestrone and progesterone administered simultaneously to increase the spontaneous activity would seem to favour abortion rather than prevent it. Some factor secreted by the placenta (not oestrone or progesterone) is responsible according to Klein for the maintenance of the corpora lutea of pregnancy and it may be that this has also some effect on uterine activity. It is obviously very important that this should be investigated in greater detail.

Summary

The behaviour of the hamster uterus in vivo has been examined. In normal animals spontaneous activity and reactivity to oxytocin are nearly the same at all stages of oestrous cycle. Spaying does not alter the behaviour of the uterus; after spaying treatment of the animal with oestrone or with oestrone and progesterone increases the amplitude of the spontaneous movements without altering the threshold to oxytocin. Adrenaline relaxes the uterus in vivo, but vasopressin is without effect.

Acknowledgments

I am indebted to Professor Hindle for the animals used and for advice on their maintenance. The purified Pitocin was supplied by Parke, Davis and Co.; the progesterone (Proluton) was given to me by Messrs.Schering. The expenses of the work were defrayed by the Rankin Medical Research Fund of the University of Glasgow.

References to Chapter Fourteen

Bruce, H.M. & Hindle, E. (1934). Proc.Zoo.Soc., p.361.

Deanesly, R. (1938). Proc.Zoo.Soc. A 108, 31.

Klein, M. (1938). Proc.roy.Soc.B. 125, 348.

Histological Note

This work was not planned for histological purposes but sections of the uteri of nearly all the animals were prepared as in previous work to see if any changes had been produced by the various hormonal treatments. As already reported by Deanesly (1938) no cyclical alteration of the endometrium in the oestrous cycle was observed, indeed the only alteration after fairly large doses of oestrone and progesterone was in the diameter of the uterus. Peczenik in personal conversation confirms that the dosage of hormones was more than adequate.

There is one very curious feature of many of the transverse sections of the uteri which was not described by Deanesly and which is as far as I know unique. In several sections there appear to be two or even three cavities in the uterine horn separated by very substantial partitions of endometrium; this partition does not however possess any smooth muscle. In other sections very blunt folds of the endometrium project more or less into the uterine cavity. The explanation would appear to be that the uterine cavity possesses very large diverticula involving the endometrial but not the muscular coat. The diverticula are too large to be described as glands; glands are present also and these are very narrow with practically no visible lumen. This unique and at first sight rather startling structure is illustrated in the accompanying photomicrographs (Fig.4).

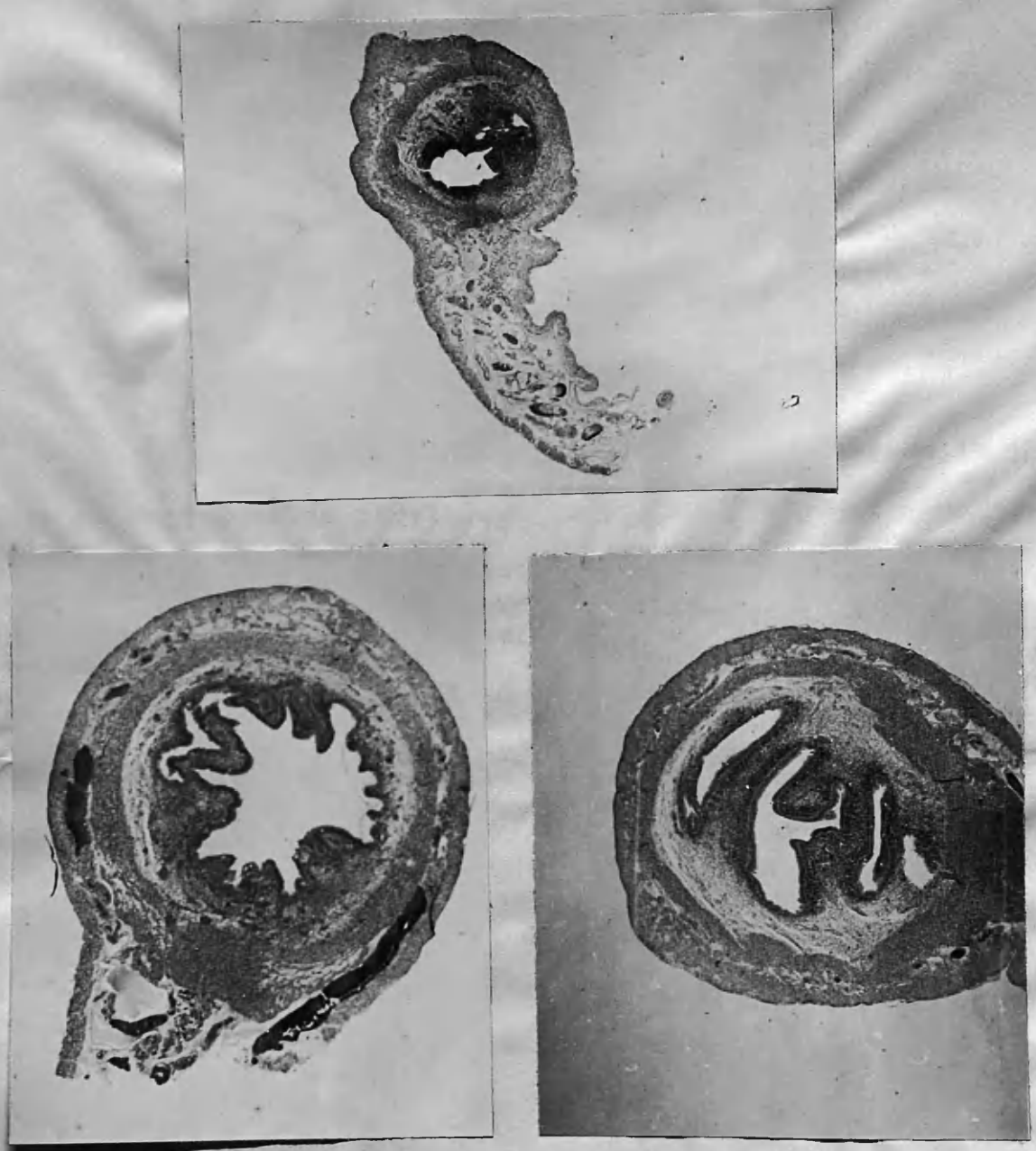

Fig. 4. Photomicrographs of sections of hamster uteri. Top: H 10, spayed. Bottom left: H 14, oestrone-treated. Bottom right: H 15, treated with oestrone and progesterone. Magnification in all cases eight times. Note the curious formation of the endometrium with very scanty glands.

Chapter Fifteen

THE BEHAVIOUR OF THE UTERUS OF THE RHESUS MONKEY UNDER THE INFLUENCE OF CERTAIN HORMONES.

From the Institute of Physiology, the University of Glasgow, 1938-1939.

In view of the well-known species differences in the realm of reproductive physiology it would be a great advantage if an animal were found whose uterine muscle reacted in a manner similar to that of the human subject. It would make investigations of dosage of hormones and of hormone therapy of uterine muscle distinctly easier. Apart from some work on the activity of the Fallopian tubes of monkeys by Seckinger & Corner (1923) in vitro, and by Westman (1929) in vivo, no investigation of the movements of the monkey uterus in vivo has been noted by Reynolds (1939) in a very comprehensive review. The present work was carried out on the most readily available monkey, the rhesus monkey. Unfortunately, well-grown specimens are difficult to get from the dealers; the animal is also rather expensive for any routine investigation. Until its possibilities have been explored, however, its usefulness cannot be defined. A review of some of the similarities and differences in reproductive phenomena between man and monkey has already been given by Hartman (1939).

Methods

The fifteen animals, all of the species Macaca mulatta, were healthy but probably subadult, except M 11, which had the wrinkled sex skin of the adult and M 3 which showed an ovulating follicle at operation although the sex skin was not wrinkled; M 3 and M 11 were the heaviest animals in the series. It is not possible to give an estimate of the age of any of the animals, but, as will be seen later, there is in the experiments no characteristic of the behaviour of the uterus which can be correlated with the body weight (as an index of the age) of the animals.

All the animals were ovariectomized through a mid-line ventral incision using aseptic precautions. Nembutal (30 mg. per kg.) given subcutaneously in about 5 c.c. of Locke's solution was found to be an ideal anaesthetic. Only a little ether by inhalation was necessary when going through the peritoneum. The abdomen was closed carefully, layer by layer, and the skin wound covered by collodion gauze. In order to prolong the period of post-operative quiescence a further dose of Nembutal was given when the animal showed signs of recovery. No further treatment was required, and the wounds healed well in spite of the great activity of the animals. No injection of hormones was given until 10 days after this operation.

The subsequent treatment of the animals is given in detail in Table I. The first group received no treatment at all,

the second oestrone in sesame oil only, the third oestrone and testosterone, and the fourth oestrone and progesterone. It will be seen that the time from spaying to the recording of the uterine movements is either 21, 26 or 32 days. In this way the diminution of the effect of the oestrone with time is allowed for, and any one group may be regarded to a certain extent as a control group to the others.

At the time of the final observations on the movements of the uterus the animals were kept under light Nembutal anaesthesia supplemented by ether during the preliminary procedures of cannulation. The abdomen was opened near the original wound and the uterus exposed; the boat-shaped cannula was attached to it by stitches in the fundus and cervix, the central stitch being taken over two pulleys to a lever writing on a smoked drum. The cannula was 2.8 cm. in length (actually the size used previously for the mouse), and because of the small size of the uterus and its fibrous character it was necessary to use a lever magnification of 35 to obtain an average excursion of 2 cm. The abdomen was closed around the funnel of the cannula; a leg vein was cannulated. The temperature was maintained throughout at 38°C.

When a satisfactory sample of the spontaneous activity of the uterus had been recorded a graded series of injections of oxytocin (specially purified 'Pitocin') was given intravenously in about 0.5 c.c. of Locke's solution followed by about 0.5 c.c.

of Locke's solution to clear out the cannula and the vein. When the threshold dose of oxytocin had been found a small dose of vasopressin (usual commercial standard of 'Pitressin', Parke, Davis and Co.) was injected. When the effect of this had passed off the uterus was quickly removed by cutting through the broad ligament and the vagina; Michel clips carrying cotton threads were clipped to the fundus and the cervix, two strips were quickly cut off and immediately suspended in oxygenated Locke's solution in 60 c.c. containers in a thermostatically controlled bath (38°C.). When the action of oxytocin had been recorded the strips were fixed for histological examination.

Results

The results of the experiments are given in detail in Table I., and typical tracings from each group are given in Figs. 1 to 4. It will be seen at once that the behaviour of the uteri within any one group is variable, and the findings in any one group overlap those in any other to such an extent that no separation is possible. The uteri of all groups showed spontaneous activity in vivo; those treated with testosterone showed perhaps less activity than the others, but even they showed occasional large waves of the extent and duration found in the other groups; the progesterone group on the whole gave slower waves than the other three groups. There was a considerable scatter of the threshold dose of oxytocin with a range of 0.01 to 1.0 unit; none

Table I. *Treatment and results*

Monkey no.	Wt. kg.	Treatment beginning 10 days after ovariectomy	Time from ovariectomy to end of exp. days	Threshold dose of oxytocin units	Vasopressin dose and effect* units	Wave duration min.	Wave height cm.	Threshold dose of oxytocin *in vitro*
M 3	4·15	None	21	0·05	—	2·0	2·4	0·5 and 1·0
M 6	2·3	None	21	0·1	0·1 R	0·8	0·5	0·2 and > 1·0
M 1	2·0	10 mg. oestrone over 7 days; no treatment next 4 days	21	0·01	—	0·6	1·8	?1·0
M 4	3·5	As M 1	21	0·3	—	0·8	2·0	?1·0
M 11	4·0	10 mg. oestrone over 10 days; no treatment next 6 days	26	0·05	0·2 N	1·4	1·0	?1·0
M 14	2·0	5 mg. oestrone over 10 days; 300 mg. testosterone propionate over next 12 days	32	0·01	0·2 small R	0·4 (6·0)	0·4 (2·0)	—
M 15	2·9	As M 14	32	1·0	0·1 R	0·8 (3·0)	0·6 (2·0)	—
M 16	2·6	As M 14	32	0·02	0·1 R	0·3	1·0	—
M 2	2·8	10 mg. oestrone over 7 days; 10 mg. progesterone over 5 days beginning last day of oestrone treatment	21	0·025	—	5·0	2·0	1·0
M 5	3·5	As M 2	21	0·5	0·1 R	6·0	2·5	1·0
M 7	2·5	10 mg. oestrone over 10 days; 21 mg. progesterone over 7 days beginning last day of oestrone treatment	26	0·1	0·1 N	2·0	1·5	0·1
M 8	1·9	As M 7 but 35 mg. of progesterone	26	> 0·05	—	2·5	3·0	—
M 10	2·0	As M 7 but 70 mg. of progesterone	26	0·01	0·1 R	0·25 (6·0)	0·2 (1·0)	?1·0
M 12	3·8	5 mg. oestrone over 10 days; 60 mg. progesterone over 12 days beginning last day of oestrone treatment	32	0·05	0·1 R	6·0	4·0	0·1
M 13	2·0	4 mg. oestrone over 10 days; 90 mg. progesterone over 12 days beginning last day of oestrone treatment	32	0·01	0·1 R	7·0	3·0	0·05

The data for the wave duration and height are average values from the first part of the tracing. The figures in brackets indicate that waves of these larger dimensions are occasional but quite prominent in the early part of the tracing.

* N = no effect; R = relaxation.

of the treatments appeared to alter the threshold. In eight out of the ten cases in which it was tried 0.1 unit of vasopressin produced a relaxation. None of the uteri showed any spontaneous activity in vitro before testing with oxytocin, but after the bath had been washed out once or twice very small movements appeared; the threshold dose was 1.0 unit or more in eight out of eleven cases. At first these findings led to the suspicion that the uterine muscle was completely unreactive in vitro; this is not so, since histamine in a concentration of 1 in a million in the bath (in the cases of M 1 and M 2) produced a contraction. Vasopressin (0.1 unit) tried out in vitro in two cases was without effect.

The histological findings (Figs.5-8) are, of course, well known and were only of interest as a check on the efficacy or otherwise of the various treatments. In the spayed untreated group the endometrium was atrophied and the glands were short and straight; in the oestrone-treated group the endometrium was thick and the glands long and curving; in the oestrone-testosterone group the endometrium was nearly as thin as in the first group and the glands were straight; the progesterone group showed the usual characteristic irregularity of the outline of the glands of the premenstrual stage - M 13 would be described as late premenstrual and the others as early premenstrual.

Another useful but not more than qualitative test of the

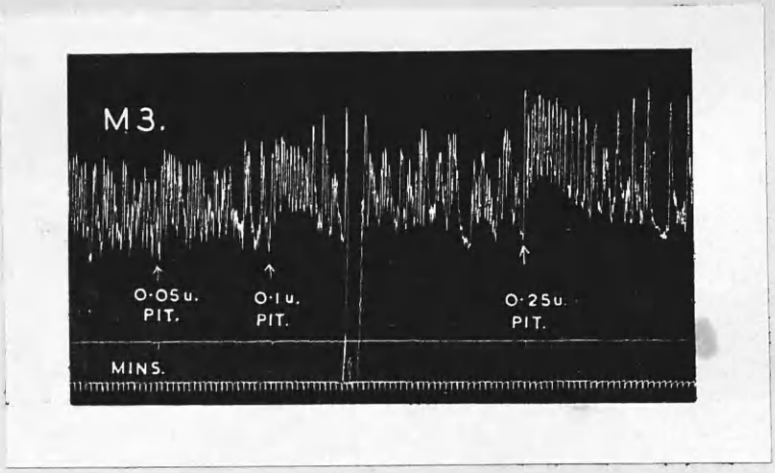

Fig. 1. M 3, no treatment. Uterine movements in vivo. Reaction to 0·1 and 0·25 unit of oxytocin, but not to 0·05 unit. The break in the middle of the tracing was accidental.

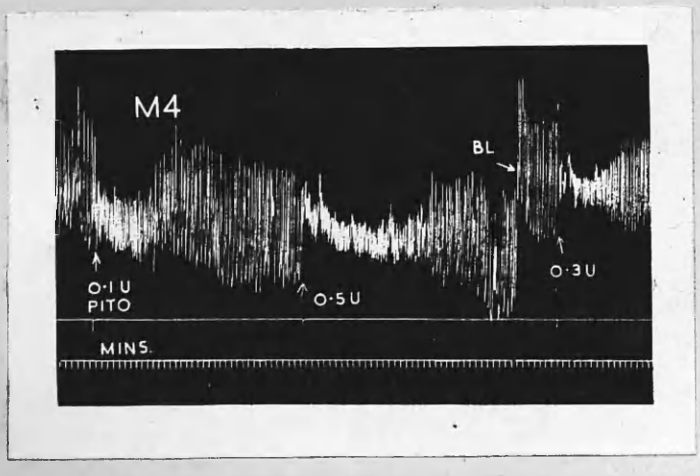

Fig. 2. M 4, injected with oestrone. Uterine movements in vivo. Reaction to 0·3 unit oxytocin. Reaction to 0·1 doubtful because there is no rise in tone. At BL the base line was raised.

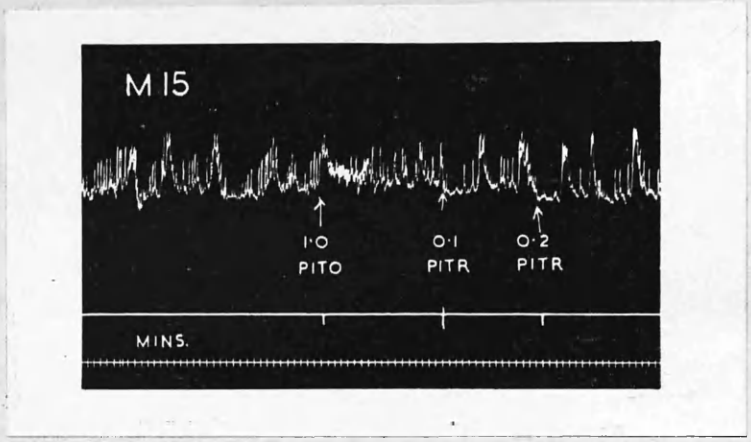

Fig. 3. M 15, injected with oestrone and testosterone. Uterine movements in vivo. Reaction to 1·0 unit oxytocin. Relaxation produced by vasopressin.

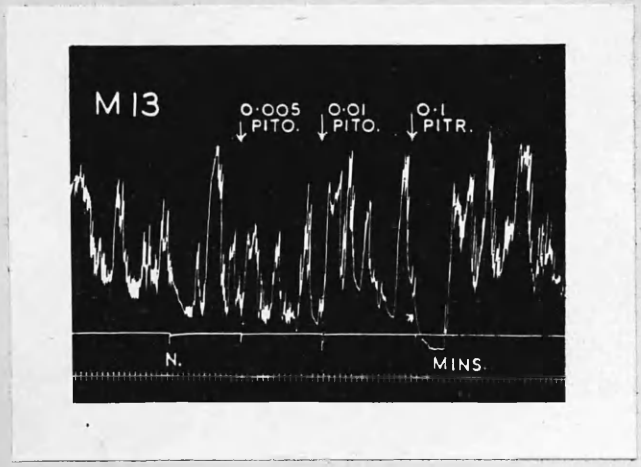

Fig. 4. M 13, injected with oestrone and progesterone. Uterine movements in vivo. Threshold reaction to 0·01 unit oxytocin. Relaxation after 0·1 unit vasopressin. At N nembutal given subcutaneously.

efficacy of the various hormone treatments was the reaction of the sexual skin. The spayed untreated animals showed no alteration of the pubic area, but all the oestrone treated animals showed at the end of the experiment a very marked oedema of this area. When only a small dose (10 mg.) of progesterone was given the sexual skin showed either very little or no swelling; with larger doses of progesterone the initial swelling produced by oestrone disappeared before the experiment was concluded. In the testosterone group the initial oedema produced by the oestrone had not quite completely disappeared when the uterine movements were recorded.

In considering the results it should perhaps be remembered that these were obtained on somewhat immature animals; the results might have been different if older animals had been used, but against this is the fact that the results obtained on the two mature animals fit in well with the others.

Fig. 5.

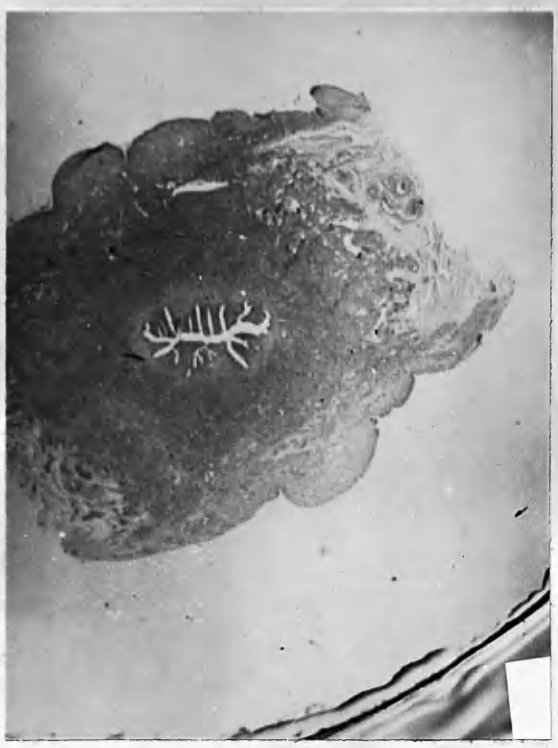

Fig. 6.

Fig. 5. M 6, ovariectomised twenty one days previously. Note the simplicity of the glands of the endometrium. Very few glands are seen in this section.

Fig. 6. M 16, ovariectomised, then treated with oestrone and testosterone. The endometrium is thin, the glands short and simple.

Magnification about eight times.

Fig. 7.

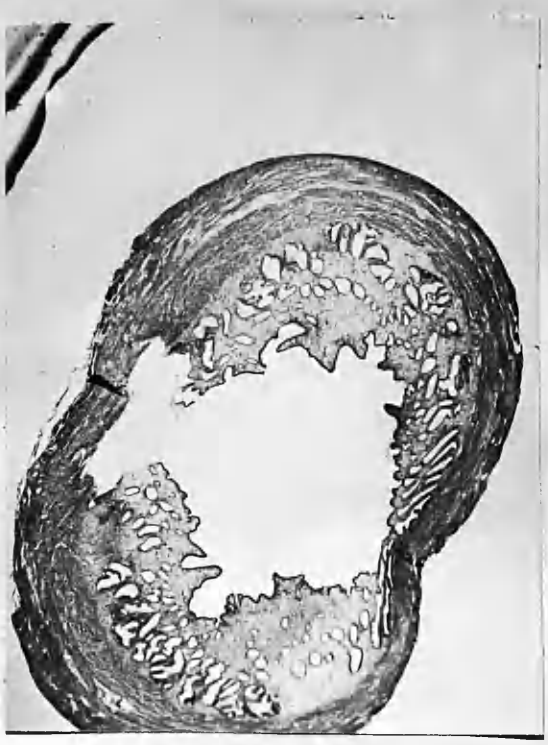

Fig. 8.

Fig. 7. M 11, ovariectomised, then treated with oestrone. The glands in the endometrium are well developed but relatively simple in shape.

Fig. 8. M 12, ovariectomised then treated with œstrone and progesterone. The endometrium is thickened and the glands are dilated and very irregular in cross section.

Magnification about eight times.

Discussion

The question of the dosage of the hormones to be given in an experiment where only a small number of experiments can be done for reasons of expense and supply had to be carefully considered. While small doses of hormones have been shown to produce endometrial changes (Hisaw & Greep, 1938) higher doses are used to produce bleeding on withdrawal of oestrone (Zuckerman, 1937a); also relatively large doses are given in human therapeutics. These considerations led to the adoption of an average dose of about 1 mg. of oestrone in oil per day; this amount should give a good contrast with the spayed untreated group. The dosage of testosterone propionate was of the same order as that given by Geist, Salmon & Gaines (1938) to women — again a large dose should produce a change if one was forthcoming. The dosage of progesterone varied over a range, on a weight basis, from about the same as that given to women threatened with abortion to a very much greater dosage.

Although there is no doubt that the hormone dosage was sufficient as judged by the endometrial and the sex-skin changes and by comparison with the therapeutic dosage, yet the activity and the reactivity of the uterine muscle were unaffected. Because there has been so much controversy as to the effects of the corpus luteum hormone on the human uterus, seven experiments of the present series were devoted to testing out the effect of a large

range of dosage of progesterone after an initial course of oestrone. In M 12 and M 13 the quantity of oestrone administered was reduced to get a higher progesterone/oestrone ratio. There is no evidence that progesterone alters the activity of the uterus or its reactivity to oxytocin. Knaus (1934) claimed that during the luteal phase of the human menstrual cycle, the uterus did not respond to oxytocin, but later workers (including Moir, 1934; Kurzrok, Wiesbader, Mulinos & Watson, 1937, and McLellan, 1940) found no alteration in the response to oxytocin throughout the cycle. The experiments of these workers and others have been criticized by Reynolds (1939) on the ground that they used a pressure within the uterus which was much above the value of 20 mm. Hg used by Knaus, and that the foreign-body effect of the intra uterine bag would be greatly increased. The present experiments in which the uterus was not distended fall into line with the majority of observations on the human uterus.

It can hardly be said that oestrone-testosterone treatment is an imitation of any known physiological process, but since testosterone has been used in the treatment of several conditions including dysmenorrhoea it would be interesting to know its effect on uterine muscle. The theory that dysmenorrhoea is produced by a muscular spasm receives some support from Moir (1936), who showed that pain and uterine contraction were related though not simultaneous, but Wilson & Kurzrok (1938) claim that patients with

dysmenorrhoea show the same type of contraction waves as those with painless cycles. In the three monkeys treated with testosterone the uterine waves were perhaps smaller and more rapid than in the other groups, but occasionally larger and slower waves were seen; it is just possible that a small reduction in amplitude might be valuable from the therapeutic point of view. It is worth while to point out here how far observations on the rabbit differ from those on the monkey; Robson (1937b) reported that testosterone made the uterus in vivo unreactive to oxytocin and produced slight progestational changes in the endometrium (see also Klein & Parkes, 1937).

The histological changes found in the present series after administration of testosterone are the same as those described for the human subject by Geist, Salmon & Gaines (1938), who suggest that the regressive changes are the 'end results of a primary inhibition of the gonadotrophic factors of the hypophysis' with consequent failure of secretion of the ovarian hormones; in the light of the present work on the monkey the endometrial changes may be due either to a direct action of testosterone or to its neutralization of the oestrone - in the intact animal these effects may be reinforced by pituitary inhibition. Loeser (1938) reported endometrial atrophy in a woman after testosterone therapy; Foss (1938) said that in his cases after testosterone the endometrium was of the interval type. In monkeys, however, previous

observers (Zuckerman, 1937b; Engle & Smith, 1939) have not reported endometrial atrophy where oestrogen treatment was followed by a course of testosterone treatment. More recently, Hartman (1940) in a comprehensive series of experiments on monkeys did not find any evidence of antagonism between oestrogen and testosterone, although the dosage and duration of testosterone treatment were not so very different from those employed in the present work.

The action of vasopressin on the non-gravid human uterus in vivo was first noted by Moir (1936) and was later worked out in a quantitative manner by McLellan (1940), who showed that the oxytocic effect of 'Pituitrin' and even of oxytocin (specially purified 'Pitocin') was due entirely to their vasopressin content. In the present experiments vasopressin almost invariably produced a relaxation; it produces the same effect in vivo on the rabbit uterus (Robson, 1937a) and on the cat uterus (Robson & Schild, 1938), but no effect on the guinea-pig or hamster uterus in vivo (see previous chapters). The human uterus seems to be an exception in its reaction to vasopressin.

The results obtained when the monkey uterus was studied in vitro furnish yet another example of the discrepancy between the behaviour of the uterus in vivo and in vitro already noted in earlier chapters on the pregnant and non-pregnant guinea-pig uterus; a similar state of affairs has been described in the case of the cat by Robson & Schild (1938). Because of this discrepancy

we cannot apply the findings of Robson (1933) on strips of the human pregnant uterus in vitro directly to in vivo conditions - this is rather unfortunate because there are only a few scattered and incomplete observations on the human pregnant uterus in vivo (Chapter One, page 69) and there is little likelihood of this gap in our knowledge being filled soon.

In spite of anaesthesia the uteri of the monkeys all showed spontaneous activity in vivo; it is rather difficult to see why they should show no movements when changed to a bath with the minimum of delay. Pregnant human uterine strips removed from women under general anaesthesia show good contractions even when there is a considerable delay in putting them into the bath of Locke's solution (Robson, 1933). Kurzrok (1938) found that some strips taken from the non-pregnant human uterus showed no activity at all and no explanation for this could be offered; he discarded all such strips as they were not 'alive'. It seems safer to regard quiescence as one extreme of spontaneous activity and to test for vi-ability by noting the reaction to stimuli (e.g. histamine) - the ability to react to a stimulus seems a more fundamental attribute of life than movement. The fact that human uterine strips in vitro usually show movements and, especially late in pregnancy, react to small doses of oxytocin, whereas the non-pregnant monkey uterine strips in vitro do not do either, is another discrepancy for which no explanation can be offered.

There is no information available about the behaviour of the pregnant monkey uterus in vitro.

Summary

The movements of the uterus of the rhesus monkey have been recorded in vivo and vitro under the influence of various hormones.

After removal of the ovaries the animals received either (1) no treatment at all, or (2) injections of oestrone in oil, or (3) oestrone followed by testosterone, or (4) oestrone followed by progesterone.

The spontaneous activity and the reactivity to oxytocin in vivo were both variable within any one group. Although the contraction waves tended to be small in group 3, and slow in group 4, there is practically no difference in the behaviour of the uteri of the four groups. Vasopressin almost always produced a relaxation.

None of the uteri showed any activity in vitro at first, and nearly all were comparatively unreactive to oxytocin although (in two cases) they reacted to small quantities of histamine added to the bath.

The similarities and the differences between these findings and the recorded behaviour of the human uterus are

discussed.

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Chapter Sixteen

THE BEHAVIOUR OF THE CERVIX UTERI IN VIVO.

From the Institute of Physiology, 1941.

It has already been mentioned in the historical introduction (Chapter One) that very little work had been done on the cervix of the uterus up to 1934. The part played by the cervix in labour is of very great importance - a considerable space is given to the physiology and pathology of this portion of the uterus in obstetrical text-books. The first stage of labour is defined as the stage of dilatation of the cervix but practically no information is forthcoming from these books about the mechanism of dilatation except that it is produced by the uterine contractions and the pressure of the membranes and liquor amnii. A certain amount of preparation for this great dilatation takes place early in pregnancy when the cervix becomes softer in consistency. It is probably not wise, and it is certainly uncritical, to assume that the cervix is simply a mechanical obstacle which must be overcome to allow the escape of the child. We have since the work of Pavlov and of Sherrington grown accustomed to the idea that both stimulation and inhibition are active processes and it may be well to keep that notion in mind in connection with the cervix. Both the alimentary and urinary tracts are provided with reflexly controlled sphincters as has been shown chiefly by

Garry and Barrington; I do not think that it is too much to expect that the genital tract should be similarly provided. This seems to be a virgin field for research - there is no mention of it in the exceedingly comprehensive review by Reynolds (1939). It is well known that irritation in the presence of haemorrhoids may produce a spasm of the anal sphincter which cannot readily be overcome; there is a good probability that the same kind of effect will be found in the cervix. Indeed the frequency with which the operation of dilatation of the cervix is performed as a therapeutic measure may be an indication that something of this nature actually occurs. Further, delay in the first stage of labour may be due to some reflex spasm of the cervix. This is all a matter for speculation at present because there is no account, so far as I am aware, of the behaviour of the cervix in normal and pathological conditions.

There is no difference, so far as ordinary histological observation goes, between the cervical and fundal muscle tissue, but when a uterus is laid open there is no doubt as to the position of the cervix on naked eye examination because of the difference in the lining membrane (see Fig.1). There is also no doubt of the pharmacological independence of the cervix and cornua in vitro as will be seen from the description of Newton's work which follows. It is very difficult to account for the lack of investigation of this structure in view of the enormous amount of work that has

Fig. 1. Post partum appearance of the guinea pig uterus. The pregnant horn was opened after fixation. The granular appearance of the interior of the cervix is quite distinct from the folded endometrium. As this illustration is about two thirds natural size it will give some idea of the size of the balloon required for cervical records free of any cornual influence.

been done on uterine movements in the past twenty years.

Newton (1934) working in University College, London, obtained from Prof. (now Sir Joseph) Barcroft samples of the cervices and cornua of goats at various stages of pregnancy (but not actually at parturition) which had been operated upon at Cambridge. These samples were received after about 3 hours delay and were placed in baths of warm Locke's solution. In the case of the cornua a small dose of oxytocin always produced a contraction of the muscle while adrenaline produced a relaxation and acetylcholine produced a contraction; in the case of the cervix, on the other hand, oxytocin even in large amounts, had no effect while adrenaline and acetylcholine both caused a contraction of the circular fibres. Newton suggested that the cervix is specifically insensitive to oxytocin during pregnancy, and that if labour is due to the action of an oxytocic substance "the advantages of insensitivity to oxytocic substances on the part of the circular cervical musculature are obvious. Similarly contractions of the cervix with relaxation of the rest of the uterus would be a co-ordinated protective movement on the part of the organ". Presumably he meant that thus parturition is facilitated and thus parturition is prevented - the cervix gains both on the swings and the roundabouts. He suggested further that this reciprocal action is mediated by the sympathetic nervous system but he was aware that the experimental findings do

not help one to speculate whether nervous or chemical influences are more important in regulating uterine activity.

Newton took up the problem again in 1937. He said that while it would be rash to say that the oxytocic principle of the posterior lobe was not concerned in parturition (as would apparently follow from experiments which have demonstrated that in some species parturition can take place in the absence of the posterior lobe of the pituitary gland) yet definite evidence that it was actually a hormone and actually involved in the process of parturition was still lacking. He stated his judgment of the matter in this carefully worded quotation which I am giving in full because of the definite attitude taken up and because the position which he regarded as unassailable may not be quite so impregnable after all.

"If such evidence (proving that the posterior lobe principle is concerned with parturition) is ever brought forward it must take account of the fact that mere contraction of the uterus is too primitive a process to be dignified by the name of parturition: it is impossible, for instance, to visualise the delivery of ten foetuses, five in each horn of the uterus, without some co-ordinating mechanism. It is not necessary to endow the oxytocic principle itself with any co-ordinating function, for the type of muscular activity might be determined by some other factor, the oxytocic principle serving merely to potentiate

the contractions. It is, on the other hand, necessary to postulate that this should not cause inco-ordinated movements.

The most severe, and at the same time the most easily demonstrable, type of inco-ordination would be a simultaneous contraction of the cervix and cornua of the uterus, and in the author's opinion this would, if brought about by the oxytocic principle, definitely settle all doubts on the score of its physiological activity".

Having made his point of view clear Newton went on to describe his experiments. He used both guinea pig and rat uteri at various stages of the oestrous cycle and in pregnancy. Cervical rings were prepared and suspended in a bath of physiological saline solution. Photographic records (magnification 175 times) were obtained. While the uterine cornua all reacted to quite small doses of Pitocin the cervix showed no alteration of activity even when 2.0 o.u. of Pitocin were added to the bath of 50 c.c. Adrenaline (1 in a million) usually made the cervix of the guinea pig contract but it had no effect on the rat cervix. The guinea pig experiments thus fall into line with the earlier goat experiments and the position of the oxytocic principle of the posterior lobe with regard to the theory of parturition is, if anything, enhanced.

On the basis of my experience in the comparison of experiments done in vitro with similar experiments performed in vivo

I had reason to doubt whether Newton's experiments gave a reliable indication of the actual physiological state of affairs in the living animal. I explained my position in the matter a few years ago to Newton and found that he had no intention of pursuing the matter further; I was therefore free to attempt experiments in vivo.

Method

It was intended from the beginning to carry out experiments in the guinea pig both in the pregnant and non-pregnant conditions. The cervix, especially in the non-pregnant animal, is a very small structure; it is not easily dilated in the non-pregnant animal but in the pregnant animal (as in the human subject) it is softer and in the parturient animal it is very soft and oedematous (Fig.1). A very small version of the tandem balloon system described by Garry (1933) for use in the rectum was soon found to be much too large in spite of great economies in construction. It was then decided to record the movements of the cornu as before by means of the boat shaped cannula and to attempt to record the movements of the cervix by means of a very small balloon placed in its canal. The next problem was to find some sufficiently delicate apparatus to record the movements. For this purpose an apparatus very similar to that shown in Fig.1 on p.155 was used in conjunction with the photoelectric amplification illustrated

in Fig.3a on p.159. Although this method provided sufficient sensitivity it was most unsatisfactory since the recording tambour was very easily disturbed by changes in room temperature or even by a draught or a slight change of pressure caused by anyone walking into the room. After many trials the following very satisfactory method was evolved.

The apparatus is illustrated in a very schematic form in Fig.2 and Fig.3. A small rubber balloon on the end of a metal cannula is tied in the cervical canal and is connected by a rubber tube to the prismatic device shown in Fig.2. The system is nearly filled with water so that the contractions and relaxations of the cervix produce corresponding changes in the level of the water in the space enclosed between the two prisms and the cover plate. These parts can be made of glass or Perspex (I. C. I. Plastics), the joints are made with Canada balsam and the whole is held together by means of a brass frame. The frame also carries a small brass tube at its lower end for connecting to the rubber tube from the cannula; the upper part of the frame carries a tube bent over on itself to exclude dust.

The prism is carried on a horizontal rod attached to the box containing the lamp and condenser which are so arranged that an image I of the filament F (motor car head lamp) is projected on to the surface of the prisms, P, P, as shown in Fig.3. When there is no water in the shaded area the light is reflected

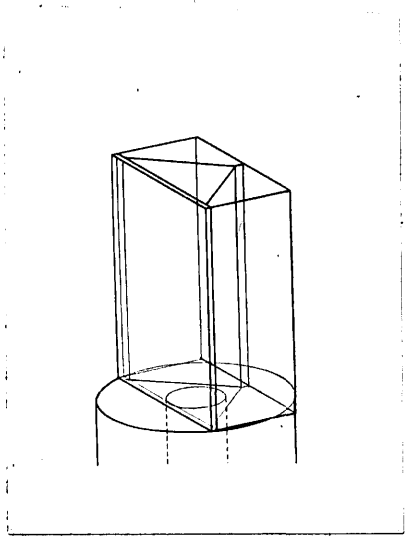

Fig. 2. Sketch of prismatic device.

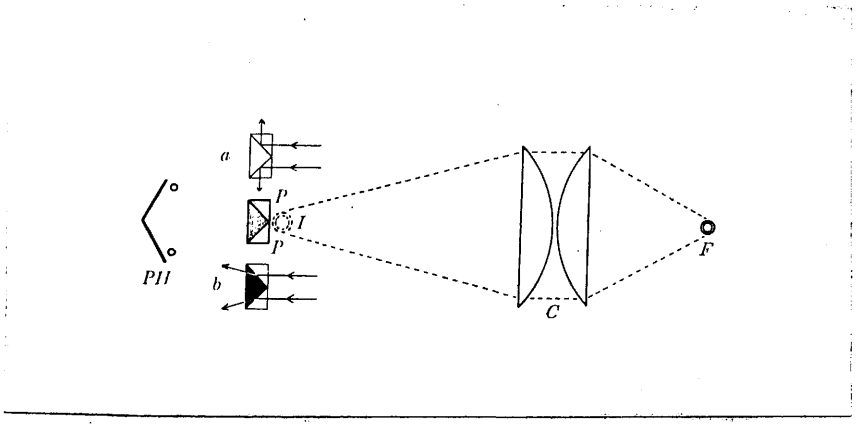

Fig. 3. Schematic diagram of the apparatus.

at the oblique surfaces (Fig.3, a) but when the water rises light passes through to the photocell, PH, as in Fig.3, b. The higher the fluid stands in the prismatic space within the range of the image of the filament the greater the illumination of the photocell (which is also carried on the horizontal rod). The movements of the fluid are recorded by a galvanometer writing on smoked paper as described in Chapter Six, pages 158-162. The apparatus is calibrated at the end of an experiment by shutting off the cervical balloon and connecting the apparatus to a calibrated pipette from which known volumes of fluid can be pushed into the prism.

It will be obvious that the sensitivity of this piece of apparatus will depend on the volume enclosed in the prismatic space; in practice it was found convenient to make this space about the same volume as the cervical balloon. The first model was exhibited at the meeting of the Physiological Society in Dundee in 1941 and gave a deflection on the smoked drum of 3 cm. for an alteration in volume of 0.01 c.c. with complete stability and freedom from temperature and draught effects. This high sensitivity has been rarely required. The intensity of the light source and the magnification of the projected image are also factors affecting the sensitivity.

Later models have undergone various modifications to give greater robustness or ease of construction. For example,

consideration of the diagram will show that a prismatic space bounded by a single prism would function as well as one bounded by two prisms. The single prism may be of water with thin glass bounding walls. Further, it is not necessary to strive for total reflection since if the beam is deflected far enough to one side when the prismatic space is empty exactly the same effect will be produced; this simplifies construction by reducing the obliquity of the prism. More recently the apparatus has been converted to a pressure recorder by closing off the upper end leaving a bubble of air to be compressed. In this way the blood pressure of the frog has been recorded and the blood pressure of small mammals should be even more readily recorded.

In certain of the experiments it was necessary to compare the effect of a quick injection with a continuous injection. A quick injection means that it is given in the usual way by means of a hypodermic syringe, the total time of injection being only a few seconds. A continuous injection, on the other hand, lasted in these experiments about twenty minutes and may be considered to be a more "physiological" procedure, i.e. the secretion of a gland may be supposed to be a slow continuous process more readily imitated by methods of continuous injection. As the method is by no means new to physiology and the variations of design of apparatus are legion it is only necessary to say that a 10 c.c. syringe was placed in a spring clamp; a platform on a

lead screw driven by an electric motor through reduction gearing gradually pressed out the contents of the syringe. The syringe was connected to the vein by means of a narrow rubber tube ending in a needle passed into the stopper of the non-clotting cannula described previously (Chapter Thirteen, page 299).

Results

As this work is still under way it may present a somewhat patchy and unfinished appearance. The gaps will eventually be filled in; but because of the war this may be a slow process. It has been very difficult to obtain animals and because of feeding restrictions the animals which have been obtained have not readily become pregnant. The necessity for obtaining pregnant animals is clear when it is realised that the investigation of the behaviour of the cervix just before and just at parturition is of the greatest importance to the theory of parturition. This information has to be supplemented with investigations of the behaviour of the cervix in non-pregnant animals under various hormone treatments to see how far any alterations which may be found can be accounted for on a hormonal basis.

The results have been gathered together into four tables. Table I. deals with non-pregnant guinea pigs, Table II. with pregnant guinea pigs, Table III. with pregnant rabbits and Table IV. with pregnant cats. The series of 29 experiments is

summarised as far as possible in Table V. It will be seen that under the conditions of these experiments the cervix shows spontaneous movements - these are in all cases measured at the beginning of the experiment before any drugs have been introduced into the circulation. The cervical movements are more frequent (i.e. of shorter duration) than the uterine movements. In nearly all cases it was possible to determine the threshold dose of oxytocin (purified Pitocin) required to produce a contraction of the cervix; this is in direct and important contrast to the experiments in vitro where the cervix was unreactive (Newton, 1934 and 1937). There is a distinct tendency for the threshold dose for the cervix to be several times above the threshold dose for the cornu. In addition the effect on the cervix of a given dose is much shorter lived than the effect on the cornu; for example, in the case of CB 39, the rise of tone after the intravenous injection of 0.01 o.u. of Pitocin was 3 min. in the case of the cervix and 20 min. in the case of the cornu. To explore this question further "continuous" injections were given to see if this would demonstrate the difference more clearly. A "continuous" injection has been defined in the description of the methods used at the beginning of this chapter. In the case of CBR 8 0.67 o.u. Pitocin was given in 10 c.c. of Locke's solution and injected slowly so that the total time of injection was 22 min. There was a great increase of tone of the cornu which lasted about an hour, the

Abbreviations: C = Contraction; R = Relaxation; I = Inhibition; T = Tone;
C-R = Contraction followed by relaxation; N = No effect.

Number Weight Date	Treatment	Extent and period of Spontaneous Movements		Threshold dose of Pitocin in o.u.		Effect of Adrenaline. Dose in microg.		Effect of Acetylcholine Dose in microg.		Effect of Pitressin. Dose in u.
		Cornu	Cervix	Cornu	Cervix	Cornu	Cervix	Cornu	Cervix	
CB 1 650 g. 7/11/40	Spayed. Then 1 mg. oestradiol di-propionate 9 days before expt.	1 cm. 30sec.	Nil	0.01 C	0.07 N	-	-	-	-	-
CB 2 530 g. 20/11/40	Spayed. Then 1 mg. oestradiol di-propionate 22 days before expt.	0.5cm. 20sec.	6mm. 1 min.	0.1 N	0.1 N	30 R	30 R	-	-	-
CB 5 360 g. 30/12/40	Spayed. Then 7 days 0.05 mg. oestrone in oil.	7 cm. 10min.	very small	0.01 C	0.02 N	100 N	100 N	-	-	2.0 u. C.of Cornu N.on Cervix
CB 6 - 2/1/41	As CB 5 followed by 3 days of 5 mg. proges-terone.	1.5cm. 3 min.	Nil	0.002 C	N	10 R	10 N	-	-	-
CB 8 350 g. 6/1/41	Spayed. 7 days of 0.02 mg. oestrone in oil.	7 cm. 4 min.	2mm. 2min.	0.006 C	0.025 C	50 N	50 N	50 N	50 N	
CB 10 277 g. 8/1/41	As CB 8, followed by 2 mg. progest-erone on 3 days.	Nil	Nil	0.005 C	N	100 N	100 N	10 I	N	-
CB 11 282 g. 9/1/41	As CB 10	11 cm. 1.3 min.	1mm. 1 min.	0.03 C	0.09 C	100 N	100 N	25 N	25 N	
CB 13 431 g. 15/1/41	Spayed. 0.05 mg. oestrone for 7 days, 5 mg. pro-gesterone for 3	Nil	Nil	0.03 C	0.7N	30 N	30 C	30 N	30 N	

Double line across Table indicates that from CB 38 onwards, the cervical records were made with the prismatic apparatus. Previous work was done by means of a tambour.

Number Weight Date	Treatment	Extent and period of Spontaneous Movements Cornu	 Cervix	Threshold dose of Pitocin in o.u. Cornu	 Cervix	Effect of Adrenaline. Dose in microg. Cornu	 Cervix	Effect of Acetylcholine Dose in microg. Cornu	 Cervix	Effect of Pitressin. Dose in u
CB 14 407 g. 16/1/41	As CB 8	6 cm. 6 min.	1 cm. 6 min.	0.03 C	0.1 slight C	30 ?C	30 N	-	-	-
CB 38 404 g. 3/7/41	Many large follicles in; vagina due to open next day.	Nil	0.001c.c. 2 min.	0.005 C	0.03 C	30 N	10 C	-	-	0.1 Cornu C Cervix N
CB 39 471 g. 10/7/41	Spayed 3/7/41, then 1 mg. oestradiol dipropionate.	2 cm. 15min.	0.005c.c. 1 min.	0.01C Rise of T. for 20 min.	0.01C Rise of T. 3min.	15 ?C	10 C	50 N	50 N	0.1 C.of both
CB 40 521 g. 11/7/41	Vagina due to open to-day. Large follicles, no corpus luteum.	1.5cm. 13min.	0.0005c.c. 1 min.	0.01 C	0.01 C	10 R	10 R	10 N	10 N	-
CB 43 560 g. 11/9/41	As CB 40.	0.3cm. 2 min.	0.001c.c. 2 min.	0.01 C	0.03 C	10 R	10 C	10 C	10 N	
CB 44 575 g. 12/9/41	7 days after opening of Vagina. Large corpus luteum in one ovary; follicles in the other.	1 cm. 1½min.	0.001c.c. 1½ min.	0.01 C	0.03 C	10 R	50 N	30 N	30 N	-
CB 46 568 g. 29/9/41	7 days after opening of Vagina.	1 cm. 1 min.	0.003c.c. 40 sec.	0.1 C	0.1 N	10 R	50 small R	10 N	10 N	-

Abbreviations as before. C.I. = Continuous Injection - for particulars see text.

Number Weight Date	Stage of Pregnancy	Extent and period of Spontaneous Movements Cornu	Cervix	Threshold dose of Pitocin in o.u. Cornu	Cervix	Effect of Adrenaline Dose in microg. Cornu	Cervix	Effect of Acetylcholine Dose in microg. Cornu	Cervix	Effect of Pit-ressin. Dose in u
CB 42 497 g. 7/8/41	Parturition previous night. Litter of 2.	1.8cm. 2 min.	0.03c.c. 3 min.	0.03 C	0.03 ?C	30 R	30 C	-	-	-
CB 46a 679 g. 26/9/41	Parturition previous night. Litter of 3.	Nil	Nil	.03 N	0.02 C	-	-	-	-	-
CB 48 667 g. 17/12/41	Parturition previous night. Litter of 1.	4 cm. 5 min.	0.01c.c. 40 sec.	-	-	-	-	-	-	-
CB 49 422 g. 22/12/41	36 hours after litter of 2 delivered.	2.5cm. 3½min.	0.04c.c. 6 min.	0.01C	0.02C	10 R	10 I 30 C-I	30 N	30 N	0.01 C.of both
CB 51 500 g. 6/2/42	30 min.post partum. Litter of 3.	Nil	Nil	0.03 C	.003 C	10 R	10 N			
CB 53 450 g. 3/6/42	5 hours post partum.	2 cm. 6 min.	0.015c.c. 2½ min.	0.002 C C.I. v.i.	.005 C	160 R C.I.	160 C	-	-	-
CB 54 600 g. 10/6/42	5 hours post partum.	1 cm. 7 min.	0.04c.c. 1 min.	0.01 N C.I. v.i.	.01 small C	300 N estimated from continuous injection	300 R			
CB 55 600 g.	55-60 days pregnant. 2 foetuses in one horn, each 12.5cm. long.	Nil	0.02c.c. 2½min.	.001 C	.03 small C	60 N	60 N	-	-	-

III. PREGNANT RABBITS

Abbreviations as before. F.T. = Full Time.

Number Weight Date	Stage of Pregnancy	Extent and duration of Spontaneous Movements		Threshold dose of Pitocin in o.u.		Effect of Adrenaline Dose in microg.		Effect of Acetylcholine Dose in microg.		Effect of Pitressin Dose in u
		Cornu	Cervix	Cornu	Cervix	Cornu	Cervix	Cornu	Cervix	
CBR 5 1.85 kg. 21/1/42	11 hours ±7 hours post partum. 3 F.T. foetuses	5 cm. 1 min.	0.06c.c. 30 sec.	0.01 C	0.03 C	3 C	0.3 C-R	30 C	10 R	
CBR 6. 2.6 kg. 9/2/42	13 ± 7 hours post partum.	3 cm. 2 min.	0.02c.c. 25 sec.	0.01 C	0.03 C	1 C	3 C	10 C	10 C	0.03 C.of Cornu N. on Cervix
CBR 7 2.2 kg. 4/3/42	Intrapartum. 2 foetuses born, 1 still in utero alive.	2 cm. 1½min.	0.06c.c. 55 sec.	less than 0.03 C	0.03 C	5 I	20 C-R	-	-	0.05 C.of both
CBR 8 2.1 kg. 20/5/42	Parturition previous night.	3 cm. 35 sec.	0.06c.c. 55 sec.	0.03 C estimated from C.I.	0.09 C	40 R estimated from C.I.	40 C-R	-	-	-
CBR 9 2.5 kg. 15/6/42	Parturition previous night.	6 cm. 1½min.	0.01c.c. 40 sec.	0.003 C	0.02 C	10 C-R	30 C	-	-	-

IV. PREGNANT CATS

Number Weight Date	Stage of Pregnancy	Extent and duration of Spontaneous Movements		Threshold dose of Pitocin in o.u.		Effect of Adrenaline Dose in microg.		Effect of Acetylcholine Dose in microg.		Effect of Pitressin Dose in u
		Cornu	Cervix	Cornu	Cervix	Cornu	Cervix	Cornu	Cervix	
PC 1 2.6 kg. 11/4/41	7 hours ± 7 post partum	Nil	0.01c.c. 30 sec.	0.01 C	0.01 C	50 R	50 C	50 N	50 N	

Abbreviations as before. Number in brackets mean number of cases, e.g. C(3) means a contraction obtained in 3 cases.

Number in Group	Category	Extent and period of Spontaneous Movements		Threshold dose of Pitocin in o.u.		Effect of Adrenaline		Effect of Acetylcholine	
		Cornu	Cervix	Cornu	Cervix	Cornu	Cervix	Cornu	Cervix
9	I. Guinea Pigs treated with oestrin, or those with ovarian follicles	2.8 cm. 6'20"	.002 c.c. 2'10"	0.011 C	0.034C (average of 6) N (3)	N (5) R (3)	C (3) N (3) R (2)	N (3) C (1)	N (4)
6	I. Guniea Pigs treated with oestrin and progestin, or those with corpora lutea	2.4 cm. 1'40"	.002 c.c. 1'	0.03 C	0.06C(2) (2) >0.1	R (3) N (3)	N (4) C (1) R (1)	N (4) I (1)	N (5)
7	II. Parturient Guinea Pigs	1.6 cm. 4'40"	.019 c.c. 2'40"	0.018C (2) >0.01	0.015 C	R (4) N (1)	C (2) C-I (1) R (1) N (1)	N (1)	N (1)
1	II. Pregnant Guinea Pigs near term	Nil	0.02 c.c. 2'30"	0.001C	0.03 C	N (1)	N (1)	-	-
5	III. Parturient Rabbits	3.8 cm. 1'20"	0.04 c.c. 40"	0.017C	0.04C	C (2) I (1) C-R (1) R (1)	C-R (3) C (2)	C (2)	R (1) N (1)
1	IV. Parturient Cats	Nil	0.01 c.c. 30"	0.01C	0.01 C	R (1)	C (1)	N (1)	N (1)

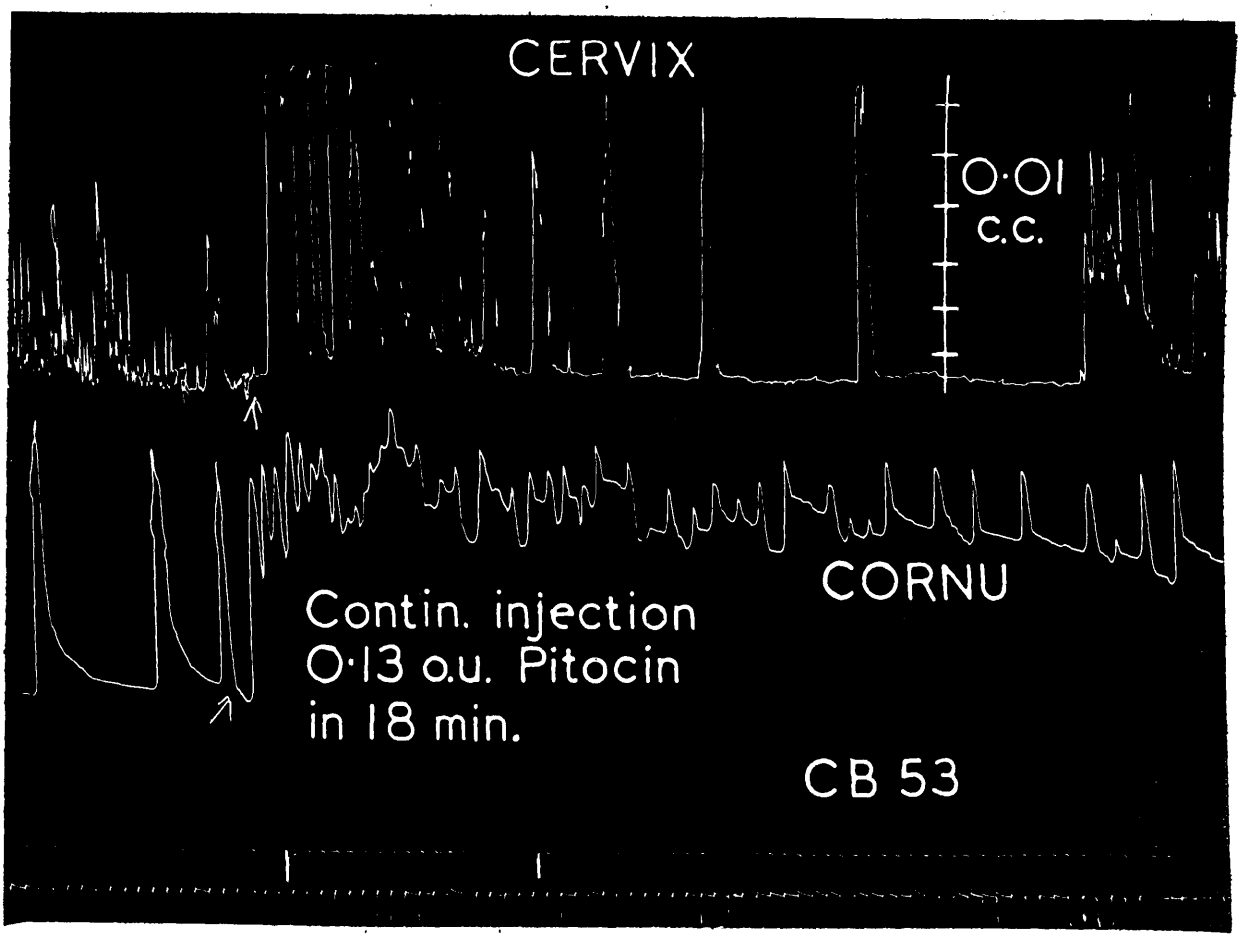

Fig. 1. CB 53. Guinea pig five hours approximately post partum. Upper curve gives cervical movements with calibration scale in hundredths of a c.c. superimposed on the right. The lower tracing shows the movements of the cornu. A continuous injection was begun at the moment indicated by the arrows - the time taken is shown by the interval between the two signals. It will be seen that the spasm of the cornu has not passed off more than forty minutes after the injection had ceased whereas the rise of the tone of the cervix is very short lived and the spontaneous activity thereafter relatively small.

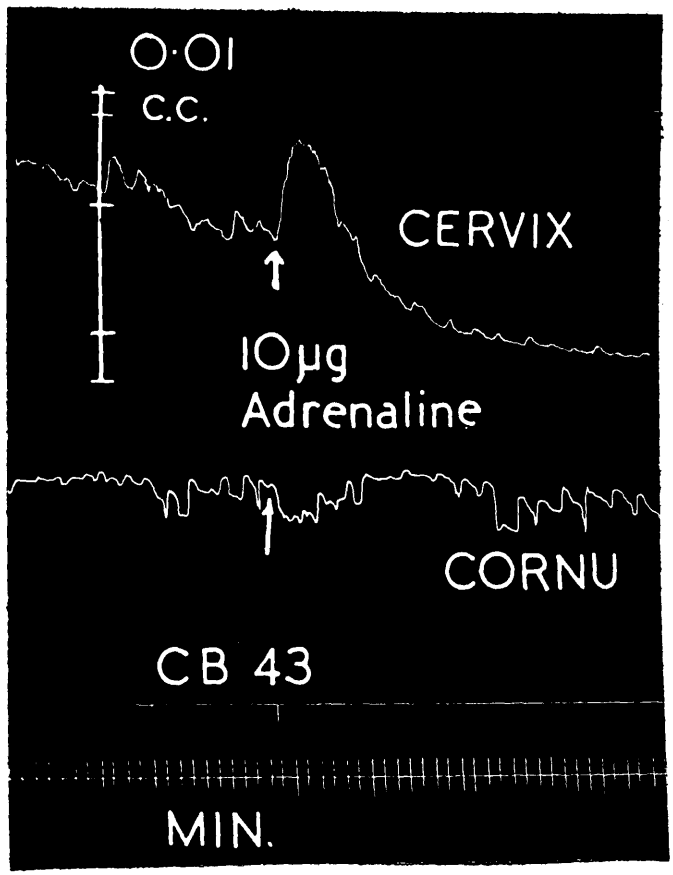

Fig. 2. CB 43. Guinea pig near oestrus. Upper tracing cervical movements, lower tracing cornual movements. There was a slight leak in the cervical recording system which accounts for the fall of the tracing to the right. 10 microg. of adrenaline intravenously produced a contraction of the cervix and a relaxation of the cornu.

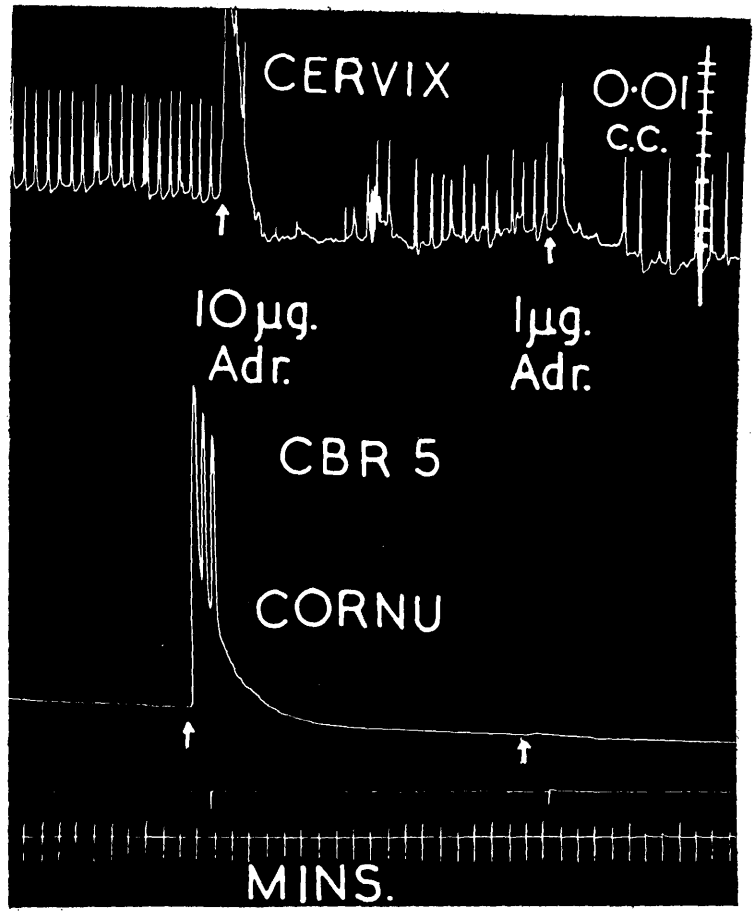

Fig. 3. CBR 5. Rabbit, 11 hours approximately post partum. The tracings show the effect of intravenous injection of 10 microg. and later of 1 microg. of adrenaline. The cervix shows a contraction followed by a relaxation in both cases; the larger dose produced a contraction of the cornu.

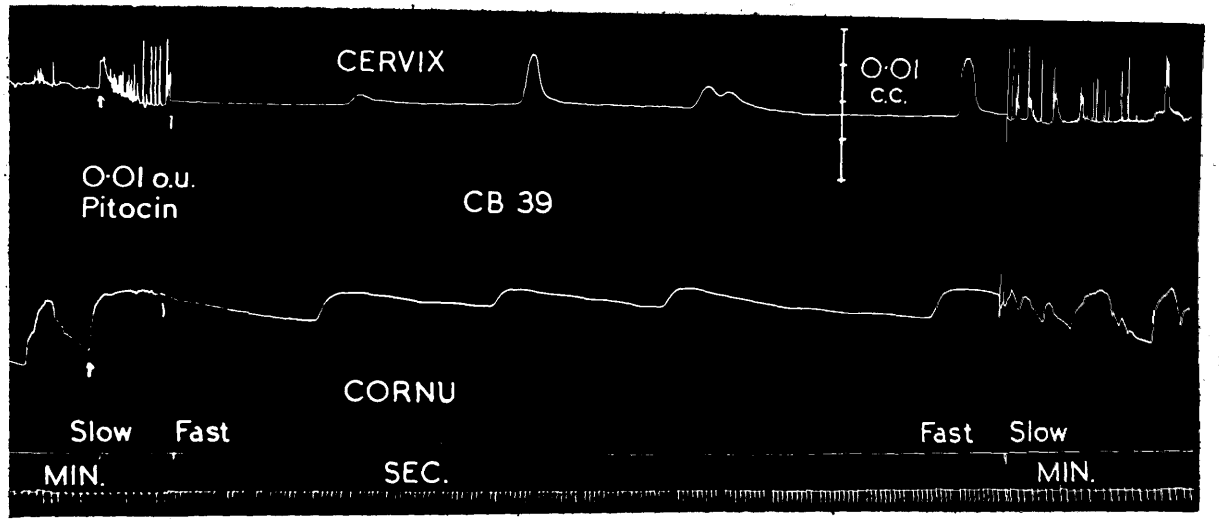

Fig. 4. CB 39. Guinea pig spayed then treated with 1 mg. oestradiol dipropionate. This tracing (of which the middle portion has been recorded at a much faster speed than the remainder) shows very clearly the different effects on the cervix and body produced by a dose of oxytocin.

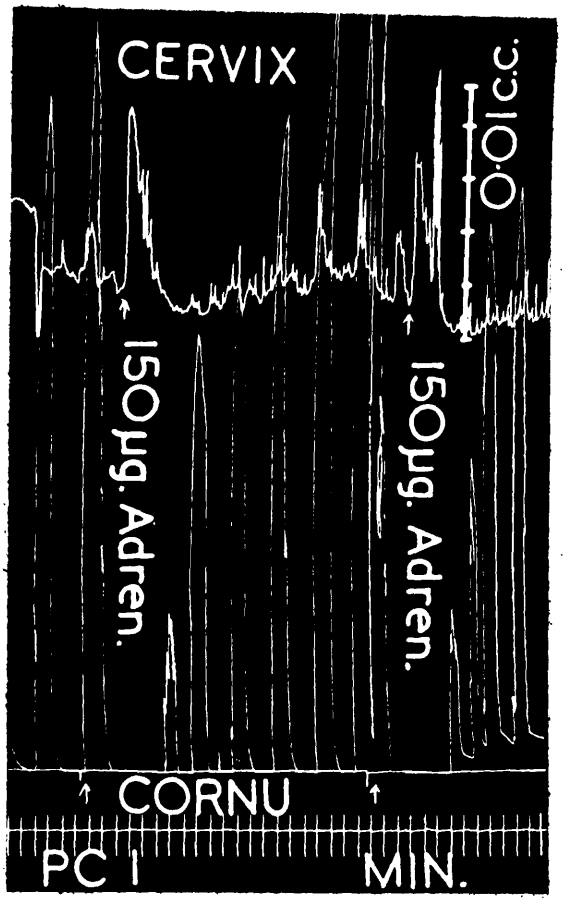

Fig. 5. PC 1. Pregnant cat about seven hours post partum. This tracing illustrates "reciprocal activity" of the cervix and cornu after a dose of adrenaline. The same dose was given twice to eliminate the possibility of a chance result. The fact that the cervix contracts while the cornu relaxes is good proof of the pharmacological independence of the two parts.

effect on the cervix was over in about 15 min. The same animal was given 0.2 o.u. Pitocin in 17 min.; the cornu was stimulated for about 36 min. whereas there was practically no effect on the cervix. CB 53 was given 0.13 o.u. Pitocin in 18 min.; the cornu showed increased activity for one and a half hours whereas the effect on the cervix was over in about 18 min. and thereafter, for about three quarters of an hour, the cervix was less active than before the injection (Fig.1, see also Fig.4). To summarise, the cervix reacts to Pitocin by contracting — the threshold dose is higher than that for the cornu and the effect of a given dose is much shorter lived on the cervix than on the cornu.

Newton found that adrenaline usually contracted the cervix in the goat and guinea pig whereas there was no effect on the cervix of the rat. In the present experiments in vivo the result of the intravenous injection of adrenaline is very variable, contractions and relaxations being produced in about equal numbers. The cervix of the parturient rabbits (Table III.) always contracted with adrenaline although in some cases this was followed by a relaxation (Fig. 3). The usual effect of adrenaline on the cornu is a relaxation but this time the parturient rabbits show either a contraction or a relaxation. In view of the variability of the responses it is not surprising to find that there were only a few uncomplicated instances of "reciprocal activity" (if the cases showing a contraction followed by a relaxation are excluded) as

found by Newton, viz. CB 43, CB 42, CB 53 and PC 1. (See Figs. 2 and 5).

Acetylcholine had usually no effect on the activity of the cervix or cornu. It ought to be stated, however, that this drug was administered towards the end of the experiments when the effects of pitocin and adrenaline had been investigated and accordingly not much stress ought to be laid on the absence of effect.

It is only in the case of the guinea pig that one can so far make a comparison between the pregnant and non-pregnant conditions. The cervix in the pregnant animals is much larger and accordingly the amplitude of its movement is about ten times that of the non-pregnant cervix. The behaviour of the parturient and also of the near term cervix is very like that of the cervix of oestrin treated animals; the period of the waves is generally longer and the oxytocic threshold is usually lower than is found in the progestin treated group. In some very recent experiments not included in the tables it has been found that two spayed rabbits treated with 1 mg. of oestradiol dipropionate showed an increase in the size of the cervix with a consistency very similar to that seen at parturition; a contraction of the cervix could, however, not be obtained even when 1 o.u. of purified pitocin was injected intravenously. Experiments on two cats, one treated with 0.5 mg. oestrone and the other with 3 mg. of oestradiol dipropionate showed very little increase in size of the cervix; indeed even in the second case it was very difficult to introduce the cervical balloon. The cervix in both cats was completely unreactive.

Discussion

There is now no doubt that the cervix uteri can be made to contract in the living animal under the influence of the oxytocic principle of the posterior lobe of the pituitary. The question immediately arises as to the position of this principle as the oxytocic agent concerned in parturition. Newton said that "the advantages of insensitivity to oxytocic substances on the part of the circular musculature are obvious" and again that "the most severe type of inco-ordination would be a simultaneous contraction of the cervix and cornua of the uterus". The work which has been described in this chapter shows, however, two mitigating features: first the cervical threshold is higher than the cornual and second the effect of oxytocin on the cervix is relatively transient.

In the latter part of pregnancy the uterine movements are of considerable amplitude; it may be that they are spontaneous or it may be that they are caused or augmented by oxytocin. The cervix may in this case remain unaffected. In the first stage of labour when (accepting the oxytocin theory for the sake of argument) the secretion of oxytocin increases the formation of the lower uterine segment and perhaps also the moulding of the head will be aided by the contraction of the cervix during the time that the body or cornu is showing active contractions. After a time, however, the cervix will cease to be affected by the oxytocic

principle and dilatation of the uterine os can then take place. In addition the peculiar behaviour of the cervical musculature towards the oxytocic hormone may have the effect of preventing precipitate labour and may be a good example of co-ordination rather than a severe type of inco-ordination as Newton would have it.

The value of making parallel investigations in several species is shown by the very recent experiments on the rabbit, and cat. Whereas the behaviour of the parturient cervix of the guinea pig is very like that of the cervix of the oestrin treated animal this statement does not hold for the cat and rabbit. In view of the general opinion that progestin is not concerned in parturition it is difficult to account for the discrepancy in these animals; the following up of this clue may shed light on the oestrin theory of parturition. If the uterus and cervix are prepared for parturition by oestrin then one is entitled to expect that the behaviour of both parts should be identical in the parturient and oestrin treated animal. Further speculation would be unwise at present but this discussion will indicate the lines on which further work will be planned.

It is very difficult to give any physiological interpretation of the action of adrenaline on the cervix when its effect is so variable and when no role for this substance in labour has yet been suggested. Usually adrenaline has the same

effect as stimulation of the sympathetic nerves to the part. Whitehouse & Featherstone (1923) found that when the lumbar cord is paralysed by spinal anaesthesia the cervix is contracted. This was demonstrated by the difficulty encountered in delivering the placenta through the cervix. These authors think that in the rabbit too the circular muscles of both body and cervix are stimulated to contract by the hypogastric nerves. Since in the present experiments the longitudinal muscle of the cornu and the circular muscle of the cervix were examined we would expect on the basis of Whitehouse and Featherstone's conclusions that adrenaline, or stimulation of the hypogastrics, would produce a relaxation of the cornu and a contraction of the cervix which was actually found in a number of the experiments. It is possible that the divergencies from this result are to be explained by the activity of the lumbar outflow which was not interfered with in these experiments. There are, in addition, certain anomalies in the innervation of the uterus which have not yet been worked out for all species. Sherif (1935) has shown that the hypogastric nerve in the bitch contains cholinergic sympathetic postganglionic fibres which run to the uterus. Curiously enough the effect of acetylcholine on the uterus is abolished by atropine, but this drug does not antagonise the effect of stimulation of the hypogastric nerves. It may be that other substances in addition to acetylcholine are liberated at the nerve endings. It will be

obvious that the innervation of the cervix is likely to be complex and variable and will present no easy problem.

Newton's experiments leave no doubt that the cervix and cornu when tested in vitro are pharmacologically different and independent. The question now arises as to whether this independence is produced by the physical separation in his in vitro experiments of the cervix from the remainder of the uterus. A study of portions of the heart in vitro might lead us to quite erroneous interpretations of the activity of the whole heart in situ. In vivo the activity of the body and cervix of the uterus is not very unlike - when the body contracts the cervix contracts generally, but by no means always, a few seconds later. Here is continuous series of stop watch observations on successive spontaneous movements of body and cervix: + means that the cervix is leading by the given number of seconds and - means that the cornu is leading.

+2,+4,+2,-2,-7,-2,-6,-9,-11,-11,-11,-10,-7,-7,-3,-2,-6,-5,-6,
-5,-11,-1, 0, 0,+2,+2,+3, 0,-7,-5, 0,-9,-10,-7,-16,-11,-18,-19.

The most noticeable feature is the great irregularity - an unfortunate attribute of all smooth muscle activity. Moir (1934) said that the fundal and cervical rhythms in the human subject were often quite independent. The form of the wave in the two parts of the uterus is often quite different, being usually more simple in the cervical tracing. This point is most clearly brought out

by inspection of the accompanying figures. These considerations indicate that there is a moderate degree of independence of the cervix; when one takes into account in addition the examples of reciprocal action of adrenaline and the difference in the response to pitocin one is forced to the conclusion that the cervix is not merely the end of the uterus but that it is a specially modified part of that organ with a physiological and pharmacological identity.

Summary

A method of recording very small variations in volume is described; by means of a prismatic device and a photo-electric cell immediate records on smoked paper can be obtained.

Twenty nine experiments are reported in - 1, non-pregnant guinea pigs; 2. pregnant guinea pigs; 3. pregnant rabbits and 4. one pregnant cat, in all of which simultaneous records of cervical and cornual movements were made. The non-pregnant cervix shows only small movements but in late pregnancy or just after parturition the movements are considerable (about 0.02 c.c. in these experimental conditions). The cervix reacts to oxytocin by contracting but the threshold is higher than that of the body and the effect, as shown by both quick and continuous injections, is short when compared with the effect of the same dose on the body.

Although these findings would fall at first sight into

Newton's category of a "severe inco-ordination" it may be that the motor effect of oxytocin on the cervix is not incompatible with the idea that the posterior lobe oxytocic principle is concerned in parturition. The response of the cervix to this principle may be concerned in the formation of the lower uterine segment and in the prevention of precipitate labour.

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Whitehouse, B. & Featherstone, H. (1923). J.Obst.Gynaecol.Brit.Emp. 30, 565.

Chapter Seventeen

The world is a jig-saw puzzle, the pieces of which are put into our hands, a chaos of isolated fragments, which yet could be fitted into an intelligible and even beautiful pattern, if we had the knowledge and imagination and wisdom to do it.

R.Livingstone (1941). Future in Education. Cambridge University Press.

It is a striking fact that persons untrained even in the elements of logic are usually logical enough for all the purposes of ordinary scientific research.

J.R.Baker (1942). The Scientific Life. Allen and Unwin.

Chapter Seventeen

A REVIEW OF RECENT LITERATURE - 1935 to 1942.

In this final chapter it is proposed to look for the most part into work which has been published since 1935, the closing date for Chapter One. A certain amount of ground has been covered in the discussion appended to each chapter and that ground will not be gone over again but an attempt will be made to link up with a wide range of other work to see how the problem of parturition is being tackled and to find how much further on we are in the matter. The reader is warned in advance that, although we are now in possession of many additional facts, we are still as far off the solution of the mystery as ever. There is an amazing amount of baffling new material which it is difficult to see in true perspective - it may even happen that in a few years the papers which I have chosen for discussion will be largely forgotten because of some new advance and work will be directed along quite different paths. There is one comforting thought on the field of reproduction - no new hormones have been found in the gonads for a number of years; on the other hand the number of supposed hormones in the anterior pituitary increases slowly but surely.

Among the uncertainties and disputed points in the question of the causation of parturition one thing is clear. In

the end the child is born by means of the contractions of the uterus. If we knew all the factors affecting the activity of the uterus then we should know all the factors involved in parturition. This may be a trite statement but it is certainly, at this stage of our knowledge, no simplification. It will serve, however, to keep our attention focussed on the uterus in spite of the distraction caused by the activity of the ovary and placenta and pituitary and even of the much neglected foetus itself.

The uterus shows spontaneous activity whether it is pregnant or non-pregnant. It is difficult to see the need for its movements in the virgin state unless it is that activity never arises out of nothing - it may be that smooth muscle must always be contracting although the extent and vigour of its contractions may vary from time to time. If we regard it as the chief function of the muscular coat of the uterus to expell the products of gestation then its previous activities may be almost accidental and it would not be wise to read too much into them and to assume wonderful co-ordinations where none exist. The idea which has been current since the middle of last century is that the Graafian follicle ejects the ovum into the open tubal aperture and that the activity of the Fallopian tube and the uterus carries the ovum down to its implantation site. In animals with a common corpus uteri migration of the fertilised ova may take place from one uterine horn through the common uterine cavity into the other

horn where implantation may take place. Internal migration of the ova of the pig has been demonstrated very well by Corner (1921) who points out that conditions in the sow are very different from those in the human. The ungulate blastodermic vesicle reaches about 30 cm. in length before implantation and this large bulk may make it specially suitable for transportation about the uterus; migration has not yet been proved before the tenth day while the vesicle is still minute and spherical. Warwick (1926) tied off one fallopian tube and removed the corresponding ovary. After breeding it was found that there were nearly equal numbers of foetuses in each uterine horn. Asdell (1924) has shown that such intrauterine migration is impossible in the rabbit, because there is no common corpus. One might conclude that this demonstrated very clearly that the uterus possessed a very highly co-ordinated activity, equal to, if not surpassing, that shown by spinal reflexes. Reynolds (1939) says that this migration takes place when the uterus is quiescent; but since his criteria of quiescence are under suspicion (see Chapter Ten) one has to withhold judgment on the point. In any case Reynolds' observations were made on the rabbit and may not be of general application. Danforth & Ivy (1938) have shown by marking accurately implantation sites during one pregnancy and by observing the implantation sites at the next pregnancy, that predetermined sites for implantation do not exist in the rabbit. The even spacing of embryos in the uterine cavity could be explained by differential growth - that is acceleration

of growth for a given distance around each implantation site as the result of some stimulus set up by the implantation. It is probable that more than this is involved since Bacsich & Wyburn (1941a, 1941b) have shown that the vascular architecture is only suitable for implantation at the antimesometrial site. It could well be that random movements of the uterus moved the embryos about till they reached a suitable site and once implantation had taken place differential growth would spread them out more evenly. The usefulness and purposefulness of the busy bee is often held up as a shining example; but the truth may be that he is a clumsy fellow and it is his awkward passage into and out of the flowers makes him a most effective cross pollinator. Similarly it may be that the random (i.e. clumsy) movements of the uterus serve to move the ova about without having any determining effect on the site of implantation.

It is rather amazing that considerable interference with the uterus just before parturition does not disturb the process of delivery. Rudolf & Ivy (1931) showed that in the post partum dog (18 - 72 hours) either separation of the horn from the corpus or removal of the utero-vaginal ganglion did not interfere with the normal co-ordinated activity of the various portions of the uterus, but the co-ordination was disturbed if both these operations were carried out. Danforth, Greene & Ivy (1937) showed that amputation of the apical portion of the uterine horn had no effect on the course of labour. There seems to be an intrinsic regulating

mechanism in each ampulla for the co-ordination of the uterine activity which evacuates it. The uterus possesses considerable resource.

The experiments of Knaus and others on the activity of the human uterus at various stages of the menstrual cycle have already been discussed in Chapter One. It will be recalled that the great majority of workers (with the exception of Knaus) found that the activity of the uterus increased as the menstrual cycle advanced. The only new papers not already discussed in Chapter Fifteen are those of Dickinson (1936 and 1937) on the technique of timing human ovulation by palpable changes in ovary tube and uterus. In his case no records were made but by the method of rectal palpation he observed the state of the uterus and appendages in a group of women in good health. There are certain advantages in such a method - there is no anaesthesia, the uterus is not distended by apparatus and repeated examinations are possible. There are certain disadvantages - the records are subjective and cannot be verified and are subject to influence of preconceived ideas (even the experienced gynaecologist may be mistaken in his diagnosis after a vaginal examination); the examination may not be sufficiently prolonged to get a typical period of activity (compare the long periods of quiescence in the pseudo-pregnant rabbit uterus in Chapter Ten); the presence of the operator and the pressure necessary to make a good examination of the parts may themselves be disturbing factors. Dickinson said that

at the time of menstruation the uterus showed moderate contractility. From about the fifth day to the middle of the cycle the uterus was quiescent and showed general and persistent firmness. From about the ninth day the uterus became increasingly active up to a climax about the 16th.day. At this time one ovary became nodular - presumably due to the formation of a corpus luteum. After the 16th.day the uterus again showed a general and persistent firmness until about two days before menstruation when the organ became more active though not to the extent seen at ovulation. This work seems to fit in well enough with that of Knaus on the human subject and that of Reynolds on the rabbit (see Chapter One); that is, it confirms their finding of uterine quiescence during the luteal phase of the cycle. Unfortunately the reactivity to oxytocin was not tested. It is difficult to comment on how far this bears out Knaus's work but from the point of view of the problem of parturition it seems to me to matter very little because the pharmacological state of the uterus during the menstrual cycle (i.e. in the non-pregnant condition) is not at all the same as at the end of pregnancy; Moir (1936) and McLellan (1940) have shown clearly that the non-pregnant uterus does not respond to Pitocin whereas Bourne and Burn (1928) have shown that the parturient uterus responds to oxytocin but not to vasopressin even in large doses. Recent experiments (Gardiner & Bradbury, 1940), however, have shown that the human uterus 6 to 9 days post partum responds equally to pitocin, pitressin or pituitrin when these substances

are administered intravenously. Bourne and Burn administered the drugs subcutaneously but it is difficult to see why pitressin by this route should not be active. This recent work raises, of course, the whole question of the validity of the method of assay of the posterior lobe extracts on the guinea pig uterus in vitro when the real intention of the experiment is to make sure that a standard preparation will be available for administration to parturient women. It is yet another condemnation added to those already given in various chapters of this thesis of work on isolated tissues. To return, however, to our original discussion, clearly it is of no value to argue from the non-pregnant to the pregnant condition; what should be investigated is the possibility of altering the state of the non-pregnant human uterus by hormonal or other means to make reactive to oxytocin. I should not be surprised if an attack on that problem lead at least to a reliable method of inducing abortion. It can hardly be said that any of the present methods is without fault.

Reynolds is the great protagonist of the inhibitory effect of progestin and therefore he strongly supports Dickinson's findings in his review (Reynolds, 1939). He attempts there to minimise the absence of any inhibiting influence of the corpus luteum hormone on the myometrium of the rat, mouse and guinea pig (see references to Siegmund and Kammerhuber at the end of Chapter One; also Chapters Six, Eight). All the work on uterine movements as Siegmund in this thesis points to the same conclusion but this was not all

published when Reynolds wrote his review. Katzenstein (1938) has measured the electrical excitability or chronaxie of the rat uterus in vitro at various stages of the oestrous cycle. If he confined his attention to the submaximal contractions only (neglecting the maximal contractions) he found that the chronaxie was lowest at oestrus (about 400 σ) and highest at dioestrus (about 1600 σ). This may be academic but it shows that there is some variation of excitability but its interpretation in terms of activity is not clear. Reynolds (1939) says that, in spite of these experiments, the uterus must be inhibited by progestin in those animals in which removal of the ovaries in pregnancy results in abortion. This is begging the question but to be fair to his side of the argument it may be that the guinea pig (for references see Chapter Twelve) is in a kind of intermediate position because in this animal removal of the ovaries frequently, but not always, results in abortion and because corpus luteum hormone tends to reduce the reactivity of the uterus to oxytocin (Chapter Thirteen) without reducing the spontaneous activity. If it were possible to get at the factor or factors in whose presence the reactivity of the guinea pig uterus is low the action of the ovarian hormones on the uteri of mice and monkeys might be worth while reinvestigating along the same lines.

Various workers have claimed that the rabbit uterus could be quietened down by the action of gonadotrophic hormones. This might explain the quiescence which sets in about five hours

or so after coitus in the rabbit (Reynolds & Friedman, 1930a). These are Reynolds & Friedman (1930b), Reynolds (1932), Sager & Leonard (1936). Morgan (1935) says that neither extracts of urine secreted during pregnancy nor anterior pituitary extract has any effect on the movements of the uterus of castrated animals; the effect of gonadotrophic extracts depends upon the effect product on the ovary. In vitro no quiescence is observed at this time (Knaus, 1934) and in vivo the effect is easily overcome by a small amount of oestrin (Reynolds, 1931; Sager & Leonard, 1936). It may be that the post coital quiescence is due to the secretion of progesterone because it is now thought that it may be released earlier than was previously imagined, that is even before the appearance of characteristic luteal tissue. It will be observed in Chapter Twelve that the threshold of parturient uterus of the guinea pig to oxytocin is high very soon after parturition; it may be that this is to be accounted for in the same way since ovulation occurs a few hours after parturition in the guinea pig although it is some days before characteristic lutein tissue can be made out. In the guinea pig (Chapter Six) injection of gonadotrophic hormones is without effect on the uterine activity of the ovariectomised animal; Evans & Miller (1936) found no preovulatory quiescence in the cow; there was considerable uterine activity for several days after ovulation but this declined from the 3rd. to 16th.day during which time the uterus was "relatively" refractory to pituitrin. It would be interesting to investigate this effect by the "unloaded"

method described in Chapter Ten where considerable doubt was cast on the inhibitory effect of progesterone. It is not unlikely that the rabbit is once more an exception to all rules. When dealing with the pituitary hormones moreover one has always to remember that they are all protein in nature and the purity of the preparation cannot be precisely stated; in addition these extracts may possess non-specific effects when injected into animals of species other than that of the source.

In view of the fact that in many animals including man pregnancy can persist even if the ovaries are removed quite a number of people have interested themselves in the idea that the placenta may take over ovarian function in these cases. In the rat it has been shown (Haterius, 1935 and 1936) that if the ratio of placental tissue to foetal tissue is high then the pregnancy will proceed even if the ovaries are removed. Haterius removed all but one foetus leaving all the placentae; although pregnancy was maintained after ovariectomy up to full term parturition did not take place. It is not so easy to see why the single foetus was not expelled especially since the experiments of Newton (1935) - already described in Chapter One - where all the foetuses were destroyed, leaving only the placentae, showed that, in the mouse at least, parturition is not interfered with by removal of the foetus. Another discrepancy is that in contrast to the artificially monotocous rat normally monotocous spayed animals (e.g. woman) can carry out parturition quite successfully. In the rat Hain

(1934) has reported that oöphorectomy performed as late as the expected day of delivery will delay parturition for one or two days. The mystery is still further deepened since the uterine musculature is not by any means quiescent at the expected time of parturition. Haterius (1936) pointed out that if the single remaining foetus were left in utero more than two days past the normal date of labour it was literally crushed to death. It might be helpful to study the behaviour of the cervix in these circumstances. An interesting extension of this work will be discussed nearer the end of this Chapter where the factor of distension will be gone into.

If the placenta is the course of progestin then analysis might be expected to give useful information - but in fact neither the human placenta nor the human corpus luteum contains much progesterone. Small quantities of progestin have been found in the human placenta by Ehrhardt (1934), Adler, de Fremery & Tausk (1934), McGinty, McCullough & Wolter (1936), Ehrhardt & Fischer-Wasels (1936), and Smith & Kennard (1937). The large amount of oestrin in the placenta interferes with progestin assays. Ehrhardt & Fischer-Wasels said that the corpus luteum hormone content of the placenta was very low indeed till about the fourth month. 72 p.c. of extracts from placentae of the 6th. to 8th. month gave a corpus luteum hormone effect; in full term placentae very little hormone was present. Pratt (1936) found that hog corpora lutea contained about 1 unit in 20 g. (see also p.132),

whereas 75 g. of human corpora lutea gave only a weak positive result when tested on the rabbit.

It is most important to distinguish between actual secretion and mere storage and therefore more attention has been given to the metabolism of progesterone since Venning (1937) and Venning & Browne (1937a, 1937b and 1938) described a method for the isolation of a pregnanediol glucuronate compound from human pregnancy urine and showed its close association with the luteal phase of the menstrual cycle of non-pregnant women. They also showed that it could be recovered from the urine to a variable extent after injection of progesterone; they suggested that the excretion of pregnanediol is possibly dependent on - 1. state of the endometrium, 2. the amount of oestrin present, 3. efficiency of the glucuronide conjugation mechanism, 4. renal excretion, and 5. excretion of pregnanediol in a form other than the glucuronidate. There is little wonder then that the results have been very variable and difficult to interpret. Browne, Henry & Venning (1937) state that the compound is present in the urine in amounts normal for the menstrual cycle (i.e. 4 to 10 mg. per litre) up to the 60th.day of human pregnancy, when it begins to rise up to 70 to 80 mg. per litre. Twenty four hours after parturition there is none in the urine. It is noteworthy that, although these American workers did not mention it, pregnanediol was one of the earliest of the sterol compounds to be isolated from pregnancy urine

(Marrian, 1929).

There has been quite a rush of work on pregnanediol excretion - the method is not so tedious as the biological methods previously necessary - Hain & Robertson, 1939; Cope, 1940; Muller, 1940; Hain, 1940; Bachman, Leekley & Hirschmann, 1940. Most of these papers do nothing more than go over the ground already covered by Venning & Browne, but Cope gives a good review of the position up to 1940. He is in general agreement with Venning & Browne although he obtained much smaller recoveries after injection of progesterone than did the American workers. Labour sets in when the pregnanediol excretion is falling rapidly and by the fifth day post partum excretion ceases completely. When the ovaries are removed during pregnancy pregnanediol excretion continues although at a lower level (Browne, Henry & Venning, 1937; Jones & Weil, 1938; Seegar & Delfs, 1940). Hain (1942) has given a very detailed account of her investigations of the pregnanediol and oestrogen excretion ascertained at frequent intervals throughout pregnancy in four women receiving treatment for recurrent abortion and in a normal woman during the last three weeks of pregnancy.

Neither labour nor abortion was associated with a rise in the excretion of free oestrogen. Rhythmic fluctuations in hormone output (of both pregnanediol and oestrogen) suggesting an extraneous control occurred at the approach of term in the three full time pregnancies and may have been present in the patient who aborted. There was no clear evidence of cyclic variations of

hormone output at monthly intervals. A large number of cases were examined in much less detail to investigate the prognostic value of hormone analysis in cases of threatened abortion. In over 60 p.c. of the cases of threatened abortion the excretion of both pregnanediol and gonadotrophins was such as occurs in a normal pregnancy. Pregnancy can proceed normally in its early stages in the temporary absence of pregnanediol output. Abortion and parturition can occur in spite of plentiful progesterone secretion supplemented even by the injection of considerable amounts of progesterone. There is no definite relationship between oestrin and pregnanediol excretion which would suggest that some oestrogenic material is responsible for parturition - in an attempted therapeutic abortion with the aid of oestrogens the pregnanediol output was high. Miss Hain sums up by saying that it seems necessary to go one step back from excretion and try to find more about secretion, utilisation and elimination. Only then will we get some real insight into the meaning of increased or decreased urinary output of this compound. The same remarks apply with as great force to oestrogen, androgen and gonadotrophic hormones.

The use of progesterone in the treatment of habitual abortion in women continues to receive support, in spite of disappointments, success having been reported with dosage of 0.5 to 5.0 mg. twice weekly. (Falls, Lackner & Krohn, 1936; Kane, 1936; Bishop, 1937; Elden, 1938; Campbell & Severinghaus, 1940; Posner & Sechzer, 1941; Mishell, 1941). The evaluation of such treatment

is an important clinical problem as well as a matter of great theoretical interest. The clinician in charge of a case of habitual abortion will prescribe rest, vitamin-E, progesterone; he might have had success with no treatment at all. Only a few papers make any effort to discuss the statistical value of the results. Further, the studies of pregnanediol excretion suggest that the progesterone excretion is relatively enormous if one supposes that, on the basis of recovery experiments, about ten times as much progesterone is metabolised as is recovered in the uterine. It is difficult to see what difference the injection of an odd milligramme per day can make on a total turnover of, say, half a gramme. In the mouse about 1 mg. is required to maintain pregnancy after removal of the ovaries (Robson, 1938a and 1938c); if allowance is made for the difference in body weight (one or two ounces against say ten stones) it will be seen that the estimate of 0.5 g. per day may not be at all fantastic. In rats 1 to 2 mg. progesterone is required daily to maintain pregnancy after oöphorectomy on the fourth day (Rothchild & Meyer, 1940); in the rabbit about 5 mg. per day are required in the later stages in addition to any progesterone which may be produced in the placenta or suprarenal cortex (Allen & Heckel, 1939; Courrier & Kehl, 1938). After removal of the pituitary in these animals similar amounts of progesterone are required to maintain pregnancy (Robson, 1936 and 1937a).

It will be seen that we are not yet in a position to

assess the importance of the hormone of the corpus luteum whether produced in the ovary, or in the placenta, or elsewhere (Cope (1940) points out that since the excretion of pregnanediol is well correlated with a progestational endometrium that might fairly enough be assumed to be the site of formation), in the maintenance of pregnancy. Several things require explanation: first, if progesterone is necessary, what does it do? It does not seem likely from the work described in previous chapters that it quietens the uterus. No one, of course, doubts the necessity for progesterone for embedding right at the beginning of pregnancy. Secondly, why the pregnanediol excretion may fall to vanishing point at an early stage of pregnancy in man without abortion occurring, and thirdly, why the corpus luteum may be removed at an early stage of pregnancy before the placenta is properly formed without terminating the pregnancy.

In those animals in which the corpus luteum is essential for the maintenance of pregnancy it is natural to enquire what it is that prolongs the life of the corpus luteum and maintains its activity. It was thought at first that the gonadotrophic principles of the anterior pituitary were responsible. Allen & Heckel (1936) found that the duration of the luteal activity in the pseudopregnant rabbit could be prolonged beyond the normal duration of 15 to 16 days by the administration of oestrin, but they believed that this was due to the stimulation of the pituitary. This theory had to be abandoned when it was found that oestrogens could maintain

the corpus luteum in the absence of the pituitary (Robson, 1937c); further than this Robson (1938b) believes that in the rabbit at least the pituitary has no direct action on the corpus luteum once ovulation has occurred. Deanesly & Newton (1941) found that if the placentae are retained the corpora lutea of pregnancy in the mouse show no reduction in size or cessation of normal growth following hypophysectomy on the 12th. day of pregnancy with destruction of the foetuses. Elimination of the placentae causes the corpora lutea to degenerate whether or not the pituitary gland is present. It is clear, therefore, that the anterior pituitary gland secretions are not concerned in the maintenance of mouse corpora lutea in the second half of pregnancy. Newton & Beck (1939) had previously shown that abortion less frequently follows hypophysectomy than ovariectomy. If then the corpus luteum is maintained by oestrin this may be supplied either by the placentae or by the ovaries themselves.

The relation of oestrin to the initiation of parturition is still a matter for debate in spite of the fact that pure crystalline preparations are now available in abundance and that they are relatively cheap so that there is every opportunity for adequate dosage. (Progesterone on the other hand is still very expensive and may not yet have been given in really adequate dosage). Experiments on the oestrin-oxytocin theory have already been discussed in Chapter One. D'Amour & Dumont (1936) have described a large study of the hormonal factors involved in parturition in the

rat which they summarise somewhat telegraphically as follows. Theory. Parturition occurs as the result of increased concentration of oestrin late in pregnancy. Results. Large doses of oestrin terminate pregnancy by killing the foetuses; doses which do not have this effect do not significantly alter the gestation period. Theory. Parturition occurs as the result of the sensitisation of the uterus to pitocin by oestrin. Results. No effect by Pitocin. Theory. Anterior lobe of the pituitary is involved either directly or by stimulating oestrin secretion in the follicles. Results. Large doses of anterior pituitary extracts delay parturition. The foetuses die eventually and are either aborted or resorbed. Smaller doses, with or without pitocin, have no effect. Theory. Some substance in the follicular fluid, placentae, or blood is responsible. Results. No such substance could be demonstrated.

Cohen, Marrian & Watson (1935) found that parturition was preceded by a fall in the total excretion of oestrogen and this has since been confirmed by a number of workers including Hain and Smith. Cohen et alii also found that parturition was accompanied by, and might be preceded by, a rise in the free oestrin content of the urine. Palmer (1938) has observed a rise in the free oestrin content of the urine at the time of abortion in a patient aborting at the 14th.week of pregnancy. Curiously enough this rise in free oestrin does not occur in mares at term (Schachter & Marrian, 1936). As mentioned earlier it is not an invariable

occurrence in woman.

Curves showing the total urinary oestrogens, oestrone and oestriol excreted throughout a menstrual cycle and in pregnancy have been published by Smith & Smith (1938), and by Smith, Smith & Pincus (1938). More oestriol is excreted during the luteal phase than during menstruation or during the follicular phase. A rise in oestrone accompanies the onset of menstruation. In pregnancy the oestriol is always higher than the oestrone after the second missed period but at the onset of labour there is a rise in oestrone and a drop in oestriol. They state that "the results in the human are in keeping with the assumption that the ovarian product oestradiol is converted into oestrone and that the degree of conversion of oestrone into the less active oestriol depends on progesterone". They suggest that both menstruation and labour may be occasioned by progesterone deficiency which no longer converting oestrone to oestriol, permits oestrone to accumulate and precipitate these effects.

Experiments on the maintenance of pregnancy by progesterone or by oestrogens are very conflicting. Bunde (1938) found that anterior pituitary extracts injected during the latter part of pregnancy in the rat inhibited parturition; heavily luteinised mulberry ovaries were produced but in spite of this two highly purified progesterone preparations did not inhibit parturition in the rat even when 3 rabbit units per day were given from the 16th. to the 22nd. day of pregnancy. Árvay (1937) found that oestrin but not

progesterone interrupted rabbit pregnancy; progesterone would not prolong rabbit pregnancy. Irradiation of the ovaries destroyed the corpora lutea without upsetting pregnancy or parturition. Anterior pituitary preparations prolonged the pregnancy of irradiated as well as of normal animals. He was inclined to believe that progesterone was of no importance in the maintenance of pregnancy. On the other hand Heckel & Allen (1938b) have shown that progesterone can prolong pregnancy in the rabbit. There seems to be no doubt that pregnancy can be prolonged by producing new corpora lutea in the rat (Hoopes, 1934; King, 1938) and in the rabbit (Snyder, 1934).

There is difficulty in assessing the importance of the excretion of oestrogens until we know more about their metabolism. There is very little information available on the amount of the oestrin in the blood and no information at all on the total oestrin content of the blood in pregnancy. Some unpublished results of Dawson & Robson (quoted by Robson, 1940) show that the blood oestrin is very low up to the 200th.day of pregnancy and only reaches measurable values in the last two months of pregnancy - equivalent to 100 microg. of oestradiol per litre.

The relation of oestrin to the initiation of parturition has also produced conflicting information in spite of the fact that pure crystalline preparations are now available. In mice crystalline preparations may not always be effective in producing

parturition even when injected in large quantities late in pregnancy. (Robson, 1935). Robson (1937b) has shown that oestrin may prolong the life of the corpus luteum in the absence of the hypophysis so that it is not to be wondered that in the rabbit injection of oestrin at the end of pregnancy may delay parturition (Heckel & Allen, 1938a). In the human being even very large doses of oestrin can be given without any effect on the pregnancy (Robinson, Datnow & Jeffcoate, 1935); oestrin administered near term is not a reliable means of induction. Oestrin is only useful in cases of missed abortion or of intrauterine death of the foetus. When oestroform (the preparation of oestrin used in these human experiments) was tried out in rabbits at various stages of pregnancy from the 9th. to the 27th. day, it was found to be actively abortifacient. It is clear from the investigations of the action of oestrin reported in this thesis that it does not invariably increase the sensitivity of the uterine muscle to oxytocin and its effect, if any, on pregnancy may have to be sought along other lines - e.g. pituitary effects, or effects on the corpus luteum.

These results may seem very confused and contradictory but reports have come in which suggest that testosterone may possess gonadotrophic activity. Hohlweg (1937) reported that luteinisation without oestrus resulted with large doses in the rat. Salmon (1938) demonstrated a gonadotrophic effect with this hormone in the immature rat ovary by means of a single injection of 1 to 5 mg. Starky & Leathem (1938) have shown that a single injection of

1 mg. of testosterone propionate produced a gonadotrophic effect on the mouse ovary. Follicle stimulation resulted but lutein tissue did not form. It has yet to be proved that these results do not occur through the pituitary but since the experiments were carried out on infantile animals it is likely that the gonadotrophic effect was a direct one on the ovary. Hohlweg & Chamorro (1937) have produced corpora lutea in infantile rats with oestrin; provided the pituitary gland was not removed too soon after the injection of oestrin corpora lutea were obtained. Since testosterone is by no means confined to the male sex these experiments may be yet another example (cf. oestrin and the corpus luteum) of the way in which the ovary controls its own activity. At the same time these results add to the difficulty of forming a clear picture of the relationships. At one time it was thought that the oestrogenic effects of ovarian extracts were unique; it is something of a shock to find it even suggested that a typical gonadal hormone is also gonadotrophic - yet another departure from specificity of action.

The review contained in Chapter One did look very favourable for the life of the oxytocin theory of parturition but interest in it has lately been revived. Fisher, Magoun & Ranson (1938) reported that labour was abnormal in cats having diabetes insipidus resulting from section of the nerve pathways between the supraoptic nuclei and the infundibulum. In all their animals

emptying of the uterus was exceedingly sluggish or failed to occur; only one animal survived in good health, and none came into oestrus subsequently so it is just possible that the anterior pituitary function may have been upset, yet several lactated normally which suggests that the anterior lobe was in fact functioning. The evidence does not warrant the rejection of the pituitary oxytocin theory of parturition. Haterius & Ferguson (1938) feel that they have saved the hormonal status of oxytocin by their demonstration that electrical stimulation of the region of the infundibular stalk of the rabbit shortly after parturition produces an unmistakable increase in uterine activity. The optimal area for electrical stimulation lies immediately above the pituitary or in the pituitary stalk and is well localised. An adequate electrolytic lesion of the stalk abolishes the response. The effect of pituitary stimulation is strikingly like that caused by Pitocin and unlike that produced by adrenaline. The response persists after spinal transection, after section of splanchnic nerves and after vagotomy. The conclude thus: "The oxytocic principle can now be classed as a hormone". A very remarkable report is made by Dandy (1940). In a young woman aged 17 a complete transection of the pituitary stalk without trauma to the base of the brain or the hypophysis was followed by normal menstrual cycles interrupted only by two pregnancies. In both pregnancies labour and lactation were entirely normal the only stigma being the continued though mild

diabetes insipidus which appeared soon after the operation; blood pressure, weight, pubic hair all remained normal. This would indicate either that the posterior lobe is not necessary for parturition in the human subject or, if it is necessary, it must be hormonally and not reflexly stimulated to secrete at parturition. Experiments similar to those of Haterius & Ferguson have been reported by a number of Chinese workers. After vagotomy and section of the cervical cord stimulation of the hypothalamus caused a delayed, prolonged rise of blood pressure in dogs (Huang, 1938) and in cats (Clark & Wang, 1939); Huang states that the effect disappeared after hypophysectomy, and Clark & Wang found the result quite different from that produced by adrenaline. These phenomena are, therefore, ascribed to the release of posterior pituitary hormones. The Chinese group have studied the release of the posterior pituitary hormones by stimulation of the central end of the vagus and find that during the pressor response the jugular venous blood contains an oxytocic substance, which is not obtainable in hypophysectomised animals, and an antidiuretic substance. The jugular blood from the isolated head was prepared by the method described in Chapter Two, and was tested on the guinea pig uterus both in vivo and in vitro. This is certainly a worth while complication of the early work carried out by me in vitro (Chapters Two, Three and Four). It is of interest that fewer positive oxytocic responses were obtained when the extract was injected intravenously and the effect noted on the living

uterus in situ than when the isolated surviving preparation was used. No attempt was made (see Chapters Two and Four) to compare the properties of the oxytocic material in jugular blood with those of oxytocin from the posterior lobe. The pressor effect gradually declines with repeated vagus stimulation and recovers with rest; these changes are correlated with the exhaustion and re-appearance of secretory granules in the pituicytes. (Chang, Lim, Lü, Wang & Wang, 1938; Wang, 1938; Chang, Chia, Huang & Lim, 1939; Chang, Huang, Lim & Wang, 1939; Sattler, 1940). These findings seem to point to the notion that both the pressor and oxytocic hormones are released into the blood stream at the same time. This would be in harmony with the opinion of van Dyke, Chow, Greep & Rothen (1942) that the posterior lobe contains a pure protein which has both oxytocic and vasopressor properties, and that after all the separation into two fractions may be artificial.

In the meantime, however, evidence has appeared (in addition to that for other animals already given in Chapter One) that even in the rabbit the posterior lobe is not necessary for parturition. In the pregnant hypophysectomised animal pregnancy can be maintained by injections of either corpus luteum hormone or anterior lobe extracts and normal parturition can occur at term. (Robson, 1936, 1937a).

The importance of the distension of the uterus by the

growth of the products of gestation has been known for a long time as a matter of ordinary clinical observation. The uterus in the case of an ectopic pregnancy in a woman is always smaller than in the case of an intrauterine pregnancy at the same stage of gestation; in animals like the rabbit in which one horn is often sterile while the other may contain several foetuses the sterile horn is very much smaller than the pregnant horn although both are subject to the same hormones since they are supplied in parallel from the same blood stream. Artificial distension with stem pessaries will bring about enlargement of the human uterus (Dickinson & Smith, 1913); distension of the uterus of the rabbit will also increase its growth. (Reynolds & Kaminester, 1936; Reynolds & Kaminester, 1937; Reynolds, 1937; Reynolds & Allan, 1937). In these experiments greased paraffin pellets (m.p. 54°) either $\frac{1}{4}$ or 1/8 in. in diameter by $\frac{3}{4}$ in. in length were used; they were inserted aseptically through the cervix by making a slit in the vagina. The endometrium and myometrium both showed increase in thickness, the greatest growth stimulus (in the case of the castrated animals) was obtained when the distending pellet was about equal to the size of the undistended uterus. These authors did not report on the size of the cervix - this is of importance from the point of view of the work described in Chapter Sixteen where it was pointed out that it was very difficult to get any growth of the cervix in oestrin treated cats and

to a less extent oestrin treated guinea pigs. For this reason it is intended to try out a combination of distension and oestrin treatment; as far as one can gather from the work of Reynolds the hypertrophy produced by distension was fairly local but this is not explicitly stated. Hammond (1935) showed that in the rabbit the uterus grows from the time of implantation to the twenty-second day and thereafter very little. Reynolds (1939) interprets this as meaning that during the period of corpus luteum activity the uterus is enabled to enlarge to accommodate the products of gestation; but that when the corpus luteum wanes and oestrin takes effect the uterus cannot then grow and so eventually would be unable to retain its contents which are meantime growing steadily. Other interpretations are easy to find: it may be that the corpus luteum and growth are contemporaneous but not more intimately related - it may be that the uterus has only a limited power of growth by increase in number of its cells and that at the time the corpus luteum is on the wane the individual muscle cells have reached a physiological maximum of hypertrophy (this principle is well recognised in unicellular organisms). But there is no doubt, in the rabbit at least, that with a greater bulk of uterine contents, that is, when the number in the litter is large the span of gestation tends to be shorter, i.e., large litters are, on the average, born before smaller ones (Hammond, 1934).

It is only recently that systematic measurements have

been made of the intrauterine tension at various times in pregnancy in the rabbit. Reynolds & Foster (1939) made a small incision into the abdominal wall under dial anaesthesia and inserted a small hypodermic needle into a gestation sac. They took as the pressure within the uterus the pressure just necessary to initiate the movement of a bubble of air in a narrow column of water towards the uterus. Measurements were made at one minute intervals to allow for contractions of the uterus and the mean was taken. The average intrauterine pressures in cm. water were 1.6, 3.06, 1.55 and 3.87 at the 16th., 22nd., 28th. and 31st.days respectively. I must say that I have never been much impressed by the magnitude of these figures. Reynolds & Foster say that only at the end of pregnancy does the pressure on the mesometrial wall, i.e. the placenta, rise to a considerable value, 3.9 cm. of water; this may be the cause of the reduction in blood flow through the placenta described by Barcroft & Rothschild (1932). Unless, therefore, the uterus empties itself the foetuses will perish by increasing encroachment on their blood supply as they increase in bulk. This all seems very neat but 4 cm. water is not a very great pressure even for a vein; if this were a determining factor it would mean that gestation would be shorter if the animal were on its back and cases of placenta praevia in the human would deliver themselves earlier provided that they were kept standing or sitting up.

The assignment of a dominant role to the changes in intrauterine tension would help us out of some of the difficulties in explaining parturition on a purely hormonal basis. It is well known however that in an ectopic full time pregnancy, with a undistended uterus, pains occur at the usual time. Other information points in the same direction. In all species so far investigated the length of gestation is not altered when the foetuses are removed and only the placentae left in situ, and this list now includes the monkey (Van Wagen & Newton, 1940). If the factor of tension on the uterine walls is of real importance then we would be entitled to expect that parturition would be greatly delayed in these experiments because almost all the increase in intrauterine bulk is due to the foetus itself; the placenta grows very little at the end of pregnancy and the liquor amnii actually diminishes. The work of Haterius & Kempner (1939) is also contrary to the expectations of Reynolds; these workers found that in ovariectomised animals bearing only one foetus with its placenta distension of the remainder of the uterus with inert pellets prevented the abortion which would otherwise have followed shortly after the ovariectomy. It seems to be that it is the distension effect rather than the hormonal effect which accounts for the maintenance of pregnancy after ovariectomy when the foetuses but not the placentae are moved (Haterius, 1935 and 1936). In no

cases where the uteri were artificially distended did delivery occur. In this connection it may be well to bear in mind the effects of tension on uterine movements discussed in Chapter Ten. It may be that the effect of the tension within the uterus while the corpus luteum is active will result in uterine quiescence and therefore maintenance of pregnancy; a further rise of pressure would then make the uterus more active towards the end of pregnancy - the decline in luteal secretion with a predominant oestrin effect would result in increasing the frequency of the uterine contractions which, when aided by a sudden outpouring of oxytocin, would bring about the delivery of the foetuses. This discussion applies only to the rabbit because the intrauterine tension has not been measured in other animals and also because the effect of load has not been studied in other species.

Friedman (1941) has made a critical re-examination of the paper of Reynolds & Foster (1939) and showed that the burden of the argument rested almost entirely upon the different values allotted to the factor of hydrostatic pressure. The equalisations in the estimated uterine wall tensions at the end of gestation depended on the making of an appropriate allowance for hydrostatic pressure in the calculations for the 28th.day and making no allowance for hydrostatic pressure on the 31st.day. Reynolds & Foster contended that intrauterine fluid had decreased to such an extent by the 31st.day that hydrostatic forces need not be considered.

This contention is not in harmony with measurements of intraperitoneal pressures; although the amount of fluid in the abdominal cavity is so scanty as to be almost negligible the intraperitoneal pressure is very definitely affected by hydrostatic forces.

Disorders of the vascular system in pregnancy have given rise to much discussion. The origin of the raised blood pressure so frequently encountered at the end of pregnancy is still a mystery. The modern work done on experimental hypertension began about fourteen years ago when Goldblatt devised his method of clamping the renal arteries. In addition there has been recently a re-investigation of the hypertensive effects of simple extracts of the kidney (so called 'renin') first described by Tigerstedt & Bergman over forty years ago. There seems to be no doubt that during pregnancy the entire vascular system is subject to some influence not operative in the non-pregnant animal. Pregnant rats are more resistant than non-pregnant rats to renin according to Harrison, Grollman & Williams (1940). These workers also find that in pregnant rats with experimental hypertension the approach of parturition is heralded by a progressive decline in blood pressure from about 170 mm.Hg. to 120 mm. Hg. starting some five or more days before the onset of labour; the blood pressure gradually returns after delivery to the previous high level. The authors suggest that the foetus elaborates an antipressor substance. Similar results have been obtained by other

investigators in the pregnant dog and rat (unpublished results quoted by Blalock, 1940). Along the same lines are the results of Dill, Isenhour & Cadden (1939); aortic constriction proximal to the points of origin of the renal vessels sufficient to produce mild hypertension in non-pregnant rabbits was found to be without influence on the blood pressure of pregnant rabbits - the pressure remained at normal levels until after parturition when it rose abruptly. The non-pregnant control animals did not appear ill at any time whereas the condition of the pregnant animals became worse. With the advent of delivery the animal at once became much more lively and within two days the animal was apparently normal. The presence of pregnancy definitely increases the susceptibility of the rabbit to renal ischaemia. Particular interest attaches to the observations of Dill & Erickson (1938, 1941) who found that an eclampsia like syndrome occurs in pregnant dogs and rabbits when the renal arteries are constricted. Within 48 to 120 hours following constriction of the renal arteries the pregnant dogs developed weakness, lassitude, coma and convulsions, and all exhibited hypertension, haematuria, albuminuria and nitrogen retention. Death occurred in 5 to 15 days, except in two cases where delivery occurred 24 and 48 hours after the onset of symptoms; liver lesions like those of human eclampsia were found at post mortem examination. In the rabbit similar results are produced but the amount of liver damage is greater. Again the

pregnant rabbit shows an increased susceptibility to the effects of renal ischaemia or renal injury. It may be argued, of course, that these changes are only incidental to parturition; on the other hand they may be pointers to some, so far unrevealed, influence of the pregnancy over the whole of the maternal organism which may in time be shown to have a bearing on the elusive problem of parturition.

In a review of the factors concerned in the duration of pregnancy by Snyder (1938) great emphasis is laid on the idea that pregnancy is composed of a series of incompletely suppressed oestrous or menstrual cycles. It is elementary knowledge that pregnancy in women lasts for ten menstrual periods and that menstruation is not necessarily completely suppressed at least in the early stages. In the mare ovulation occurs spontaneously during pregnancy; in the cow the graafian follicles in the ovary may enlarge very considerably; in the rat follicles develop at the normal times of the oestrous cycle throughout pregnancy; the ovarian activity of the guinea pig does not seem to be entirely suppressed (Chapter Twelve); Hartman (1932) said that in the case of the monkey "while the fertilised ovum is implanting itself in the uterus the factors involved in the maintenance of the sexual cycle still operate, though much dampened down, so that only one or at most two abortive cycles result, which, however, seldom attain the bleeding stage after the haemorrhage of implantation or the

placental sign has ceased". Bartelmez (1937) suggests that the same can occur in the human; all human embryologists are familiar with cases in which the embryo is obviously a month older than is indicated by the menstrual history. There is no doubt that abortion in the early stages of pregnancy in the human subject takes place most frequently at the time at which a menstrual period would have occurred in the absence of the pregnancy; women with long menstrual cycles are said to have long periods of gestation. All these observations suggest that parturition occurs at the end of a partially suppressed cycle. Although it can be readily admitted that the conditions seem most favourable at this time the idea is more a basis for further work than a real explanation of the phenomena of parturition.

This chapter contains some of the material of the jigsaw puzzle of parturition. There may be more reasons than those given in the remarks of Livingstone quoted at the beginning of this Chapter for our inability to put the puzzle together. There are still gaps in our collection. The biggest missing area deals with the relationship between foetus and mother; in all descriptions of reproductive physiology we meet the phrase 'if the ovum is fertilised' certain things happen. The action of the fertilised ovum on the endometrium, on the corpus luteum and on the pituitary are now well known but we have no real inkling as to how they are brought about. Other difficulties in our jig saw are that we may

have more than one puzzle in our collection but we have so far found no way of separating the puzzles. The problem of species difference is very troublesome but it will have to be solved before any satisfying answer to our question is obtained. As I said earlier in this thesis the search for a fundamental pattern, and for its modifications, should be the main object of our endeavours.

One of the principles on which the body works is the maintenance of the status quo - the principle of homoeostasis. But it would seem that the body is capable of more than this. Just as a bomber may get home with one or more engines out of action so it seems that parturition can be brought about in the absence of this or that hormone, or organ, in the absence of nervous connections and in all sorts of other difficulties including even the absence of the foetus. One can only conclude that in normal circumstances parturition occurs when, for reasons which are still beyond us, many things combine to support and favour that occurrence. Furthermore in considering the problem we are many times confronted with the difficulty of distinguishing accompaniments from causes.

It is not easy to summarise the effect of the work described in this thesis on current ideas on parturition when in any case the current ideas are extremely vague. What, for want of a better title, may be called the 'oestrin-oxytocin' theory

seems to be the best ground for argument. While the effect of oestrin is rather variable and makes little or no difference to the reactivity of the uterine muscle of some species to oxytocin, yet in all species of which we have information oxytocin produces a uterine contraction more easily at term than at any other time. There is some difficulty in determining the source of the oxytocic material - it may arise in the maternal pituitary or placenta or even in the foetus - and in determining when, if ever, it is present in the circulating blood. Another difficulty - in the guinea pig at least - is to account for the long period of high reactivity to oxytocin before parturition actually occurs. If parturition is to be accounted for by a sudden outpouring of oxytocin then we are entitled to enquire how this sudden outpouring is brought about. The behaviour of the cervix uteri, although at first glance, an example of a severe inco-ordination when tested with oxytocin in vivo, is not inconsistent with the oestrin-oxytocin theory. The effect of the hormone of the corpus luteum on the uteri of many species, including the rabbit, has been found to be the same - that is, there is no reduction in the extent of spontaneous activity although the rate of movement may be altered.

I am well aware that my work has been concentrated almost entirely on uterine physiology. This is by no means the whole story of pregnancy and parturition. The preparation of the mammary gland during pregnancy, the initiation and continuance of lactation

have all to be accounted for. The placental function, the transference of materials including food and hormones between foetus and mother, the formation of liquor amnii and many other problems must be fitted into a complete description. In addition the whole story of reproduction would require a description of embryology and of the dynamics of embryology. The subject is colossal and our knowledge microscopic; it is easy to prophesy that physiologists and clinicians and biochemists will be occupied with the problem for many years to come.

There seems to be some essential clue missing from our account of the problem of parturition; there is no indication as to how the present deadlock will be relieved. More and more energy, and more and more journals, are being devoted to reproductive physiology; more and more detail is accumulating. In spite of this concentration of effort every review is, like the present one, full of statements which are carefully qualified so that we are left with nothing substantial to cling to. Considerable advances were made when the chemical constitution of the gonadal hormones was elucidated. It is easy enough to prophesy that there will be astounding advances when the constitution of the pituitary hormones is as well known. This is, however, a matter of protein chemistry which is making at the moment such slow progress; fortunately chemists are becoming less and less daunted with the apparently impossible. It may be that we are waiting for the

discovery of some new hormone which will show us how the known facts can be connected together in a logical fashion; there are indications that new hormones may emerge before long. There is one serious gap in our knowledge of every hormone - we have practically no information about its metabolism; we should like to know from what materials the hormone is prepared, how fast it is released, how it acts and how it is eliminated. But these are hard questions; progress may come by some means not imagined by the prophet. Time alone can tell.

> When a man hath finished, then he is but at the beginning; and when he ceaseth then shall he be in perplexity.
>
> Ecclesiasticus, XVIII. 7.
> (Revised Version by W.O.E.Oesterley,
> Cambridge University Press, 1912).

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