WITHDRAWAL OF THE LIQUOR AMNII AND YOLK SAC FLUID FROM THE RABBITS UTERUS ITS EFFECTS ON FOETUS AND PLACENTA

BY
BENJAMIN PHILP WATSON
M.B., CH. B.

X

WITHDRAWAL OF THE LIQUOR AMNII AND YOLK SAC FLUID FROM THE RABBIT'S UTERUS ITS EFFECTS ON FOETUS AND PLACENTA

BY

BENJAMIN PHILP WATSON

M.B. , Ch.B.

TABLE OF CONTENTS

INTRODUCTION	page	1
ANATOMY OF FEMALE GENITAL ORGANS OF THE RABBIT	"	5
DEVELOPMENT AND STRUCTURE OF OVUM AND PLACENTA	"	9
METHOD OF CARRYING OUT THE EXPERIMENTS	"	24
DETAILED ACCOUNT OF EXPERIMENTS	"	30
SUMMARY AND DISCUSSION OF RESULTS	"	128
LITERATURE	"	179

INTRODUCTION

I N T R O D U C T I O N

Among the problems of development that still await solution none has occupied a more prominent place , so far as wealth and variety of investigation are concerned , than the origin of the liquor amnii. The literature on the subject is voluminous as it has been approached from many different standpoints. Some have endeavoured to arrive at a solution from observations of pathological conditions , such as hydramnios and oligohydramnios in the human subject; others have made exhaustive researches into the chemistry of the amniotic and allantoic fluids in the lower animals; others again have investigated their physical properties; while yet others have relied upon experimental work , such as the injection of substances into the mother and the recovery of them in the liquor amnii. Yet the solution of the problem seems to be almost as far off as ever.

In looking over the literature of the subject I could find no record of experiments made by the artificial withdrawal of the fluid and the observation of its effects. Such a line of investigation seemed to be a promising one , and I accordingly carried it out.

The results which I have obtained have been definite , so far as the effects on the foetus and placenta are concerned,

and as showing that no re-secretion occurred; but the first result of the aspiration of the fluid has been in all cases the death of the foetus , so that the deductions to be drawn regarding the origin of the fluid cannot be equally definite.

I have however , in my series of experiments been able to study the changes occurring in the placenta and foetus after intra-uterine death , due to an uncomplicated cause.

Thus it is that the greater part of what follows is concerned with the changes which occur in the retained placenta subsequent to the death of the foetus. I do not know of any similar observations on the lower animals. My results are interesting from the light which they throw on the pathology of the retained human placenta , and on the changes which the foetus undergoes when it is retained in utero after its death.

The animals selected for observation were rabbits. They were chosen because of the facility with which they can be obtained and bred , and also because the normal structure of the placenta has been very fully worked out , especially by Minot , Duval and Chipman. To the work of the latter I am especially indebted as giving the most minute and detailed account of the whole gestation period. In my own specimens of normal placentae I have also been able to follow out the series of changes which occurs from the 8th day on-

wards - observations which it was essential to make , in order to interpret properly the pathological appearances induced by the experiments carried out. Though a short description will be given of the normal structure of the placenta of the animals operated on , both at the time of operation and at the post mortem , it may be well here to summarise the facts known regarding the anatomy of the genital organs of the rabbit and the development of the foetus and placenta.

ANATOMY OF THE FEMALE GENITAL ORGANS

OF THE RABBIT

ANATOMY OF THE FEMALE GENITAL ORGANS OF THE RABBIT.

The rabbit's uterus consists of two cornua entirely distinct and separate from each other except at the vaginal extremities , where the apposed walls are fused for a distance of from $\frac{1}{4}$ to $\frac{1}{2}$ an inch. Each opens separately into the common vagina. At the other extremity the cornua are continuous with the Fallopian tubes , which are slightly convoluted and much narrower than the uterus proper , while they are about double the length. The division between tube and uterus is marked by the attachment of the round ligament , and by the diminution in the diameter of the tube.

The wall of the uterus proper consists of three coats , peritoneal , muscular and mucous. The peritoneal coat is continued over the uterus on to the mesometrium , constituting a suspensory or broad ligament which attaches the organ to the pelvic wall, and carries to it its vascular and nervous supply. The muscular coat consists of two layers - an outer longitudinal , and an inner circular , separated by some loose connective tissue , which contains large vessels running longitudinally. These two layers are of equal thickness.

The mucous membrane consists of a delicate sub-mucous tissue , covered by a single layer of columnar ciliated epithelium. It does not form a smooth lining to the uterus ,

but is at certain points raised into large dendritic processes which project into the cavity , almost completely filling it up. Two of these folds are specially large , and are situated one on each side of the mesometrium. It is by the hypertrophy of the connective tissue of these " placental " folds that the maternal part of the placenta is almost entirely formed. On each lateral wall are similar though smaller projections - periplacental folds - while directly opposite the placental folds are two others - obplacental folds - one on each side of the middle line , which are the smallest of all. The difference in the size of the projections has the effect of rendering the lumen of the cornu slightly excentric in position. Besides these six large and distinct projections there are innumerable smaller foldings which on cross section appear as glandular spaces and constitute the " uterine glands," while transversely running fissures divide each of the large folds into rectangular areas.

The structure of the tube is essentially the same , the only difference being the smaller diameter , the less branched condition of the mucosa , the greater equality in the size of the folds , and the realatively greater thickness of the inner , circular muscular coat. It is suspended in the same way as the uterus by the suspensory ligament.

Each ovary lies on the suspensory ligament close to the

fimbriated extremity of the tube. The structure of the vagina calls for no detailed description here.

DEVELOPMENT AND STRUCTURE

OF THE OVUM AND PLACENTA

DEVELOPMENT AND STRUCTURE OF THE OVUM AND PLACENTA.

Ovulation is said to occur as a rule a few hours after impregnation , and from three to nine ova are discharged at one time. These entering the oviduct are at once fertilised, and continue to travel down the duct into the uterus , which they reach about the third day. It is not however , till the 8th day that the ovum becomes attached to the hypertrophied maternal mucosa , which meantime has undergone important changes. The ova are spaced out at more or less regular intervals in each cornu , and as the intervening parts remain of almost the same diameter as before impregnation , the horn becomes divided up into a series of " sacs," each containing an ovum. As a rule the sacs in the two cornua are unequal in number , and all the sacs may not be of equal size , this being due to the fact that some of the ova are later in being fertilised than others. It has been stated that the younger ova are situated at the tubal , and the older at the vaginal extremity of the cornu , but Chipman found that such was by no means constantly the case , and that where differences in the stage of development were evident , the distribution along the cornu was quite irregular. In carrying out my experiments I have always been careful to select for observation and control sacs which were of equal size , and so presumably

of the same age.

While the ovum is travelling down the oviduct and uterus and during the period in which it lies unattached within the latter , it undergoes important developmental changes. Segmentation is said by van Beneden to begin some 10 or 12 hours after fertilisation , and goes on rapidly , so that by the 3rd day , when the ovum enters the uterus proper , it consists of two parts - a central part composed of large granular cells , and a peripheral part of smaller , clearer cells , which latter completely surround the former. By the end of the 4th day , by reason of the very rapid growth of the outer layer of cells , the blastodermic vesicle is completely formed. It is spherical in shape , and consists of an outer wall of flat polygonal cells , to which is attached at one point a mass of granular cells derived from the inner cells of the preceding stage. The rest of the vesicle is filled with fluid. During the 5th day the vesicle increases in size , and three layers become differentiated at the area of attachment of the granular cells , the outer being the original outer layer of the vesicle , and the two inner being derived from the inner granular mass of cells by a process of splitting. These three layers are only present at the " embryonal area," the rest of the vesicle being composed of one layer of cells. At the end of the 6th day the outer layer of cells has almost

disappeared , and the middle layer , which is the epiblast , is thicker , owing to a change in the shape of the cells from cubical to columnar , while the inner layer of hypoblast consists of flattened pavement cells.

By the 7th day the shape of the blastodermic vesicle has become ellipsoidal , the outer layer of cells has completely disappeared from the embryonal area which thus comes to consist of two layers - the epiblast , which is the former middle layer , and the hypoblast. The latter now extends about half way round the vesicle. The embryonal area is pyriform in shape , and at the posterior or narrower end there appears an axial thickening of the epiblast - the primitive streak which extends about two-thirds of the length of the area , and in the centre of which appears the primitive groove. At this stage also the mesoblast appears , spreading out as a thin sheet on each side of the primitive streak between the epiblast and hypoblast. The average diameter of the vesicle at this stage is 3.5 to 4 mm. From the 7th day onwards the development of the embryo of the rabbit differs in no essentials from that of other mammals. Thus , in front of the embryonal area the neural groove appears which is later converted into the neural tube by the uprising and fusion of the neural folds. By head , tail and side folds the embryo becomes constricted off from the rest of the blastodermic

vesicle. This is well advanced by the 9th day , by which time in addition , the embryo has become flexed on itself ventralwards. By the 10th day , by reason of the very rapid growth of the cephalic and caudal extremities , the flexion is much more marked. By the 12th day the visceral arches and clefts are developed , and the limbs , with indications of division into their several segments are distinctly visible, while the divisions of the brain are recognisable. The coelom or body cavity which arises about the 8th day by a splitting of the mesoblast , and which extends beyond the embryo proper almost to the edge of the vascular area of the yolk sac , becomes closed in completely at the 10th day.

The later stages in the development of the various organs will not be further detailed here , but will be referred to under the several experiments as occasion may demand. The origin , development and arrangement of the yolk sac , amnion and allantois however , require fuller description , as they differ in many respects from the corresponding structures in other mammals.

The Yolk Sac.

This is the extra embryonic part of the blastodermic vesicle after the embryo has become constricted off by the tail , head and side folds. It is at first a more or less round vesicle with a wall composed of two layers - hypoblast

and epiblast. In its upper part , i.e. , in the part next the placenta there is a layer of mesoblast between these two , and in this run the vitelline vessels. This is known as the vascular zone of the yolk sac; it extends almost half way round , and its limit is marked by the circularly running vessel , the sinus terminalis. The vesicle contains fluid , and at the 9th day it completely fills the gestation sac , the non-vascular part being in contact with the uterine mucosa (fig. 1). As early as the 8th day buds of epiblast can be detected springing from this part , and these serve to attach it to the uterine wall , and may be of some nutritive importance.

By the downward projection of the head of the embryo at the 10th day the upper vascular wall becomes driven downwards , and as the embryo grows and the amniotic cavity distends , this becomes more and more marked , so that at the 13th day the vascular and non-vascular parts are in contact , and the yolk sac has ceased to be a vesicle. The lower non-vascular wall has meanwhile been degenerating and becomes absorbed , so that by the 16th day only traces of it remain , and the hypoblast of the vascular part of the yolk sac is in contact with the uterine wall. Fig. 1 shows the relations of the yolk sac at the 10th day , and fig. 2 at the 16th day of gestation.

The vitelline circulation begins about the 8th day , and by the 10th day it is fully established. The vitelline arteries convey the blood to the sinus terminalis , and branches from it , and from the vitelline arteries themselves break up and end in capillaries from which the blood is collected into the vitelline veins which run parallel to the sinus terminalis but nearer the embryo , and which open into the heart. The vitelline circulation persists up to the time of parturition , but it is insignificant as compared with the allantoic in the later stages. This is a point which will be referred to more fully later.

The Amnion.

This is formed by folds from the somatopleure , which rise up and gradually enclose the embryo. Chipman has shown that the side folds take the largest share in its formation , and after them the tail folds. At 8½ days he has demonstrated by serial sections from the anterior to the posterior end of the embryo that this is so. The amnion is not completely closed over the embryo until the 10th day (fig. 1). From this time onwards it gradually becomes distended with fluid , and fills up the gestation sac , driving the vascular wall of the yolk sac before it (fig. 2).

The Allantois.

The allantois takes its origin about the 9th

day from the posterior part of the alimentary canal. It extends along the mesodermic surface of the amnion , so as to reach the mesoderm of the ectoplacental laminae , to be afterwards described. It consists of a wall of mesoblast lined by entoderm , the cavity containing fluid which increases considerably in amount from the 10th to the 12th day. The mesoderm conveys the foetal vessels to the placenta. By its extension along the mesodermic wall of the amnion the allantois comes to intervene between the former and the placenta , but the quantity of fluid it contains is small in amount as compared with the liquor amnii.

The Placenta.

The placenta consists of two parts , maternal and foetal , the former being developed from the two mesometric or placental folds of the uterus previously described , and the latter from the foetal structures to be hereafter detailed. The maternal placenta from its origin in the two placental folds is a bilobed organ , and remains such until the end of gestation.

After impregnation the sub-mucous tissue of the two placental cotyledons begins to hypertrophy by the rapid division of the connective tissue cells , so that by the 4th day they are two cushion-like structures , made up of star-shaped and spindle-shaped connective tissue cells with numerous capill-

aries between them. The surface and glandular epithelium also undergoes division , the former completely investing the free surface of the folds. Meanwhile , the periplacental and obplacental folds are undergoing atrophy , and as the placental and foetal appendages develop they go on doing so until they can be no longer recognised as distinct projections and the interior of the cornu becomes almost smooth.

At the 4th day there is a great deal of ground substance between the connective tissue cells at the placental site; this goes on increasing in amount along with the cellular elements up to the 7th day. By the 6th day the mesometric thickenings have become truncated , and they occupy a large part of the diameter of the sac , this being more apparent owing to the atrophy of the obplacental folds. At this time giant cells make their appearance in the mucosa opposite the placental site. They are of enormous size , and are derived from the surface and glandular epithelium. They persist until the 14th or 16th day of gestation.

At the 7th day there is an enormous increase in the number of vessels in the placental area. These vessels are dilated capillaries , with no muscular or adventitial coat. At this time " decidual " cells first make their appearance. They are large spherical cells with a single round or oval nucleus , and are derived from the connective tissue cells of

the corium. They are first seen in the deeper parts of the placenta next the mesometrium , and are developed round the dilated capillaries , constituting perivascular sheaths.

The epithelium on the surface of the cotyledons has undergone rapid division , so that the cell outlines are lost and the tissue becomes of a plasmodial character with numerous nuclei scattered throughout. The glands still persist , and their cells show no such tendency , though their mouths are often blocked by the hypertrophy of the surface layer.

By this time - the 7th day - the embryonal area is evident in the blastodermic vesicle , and the latter occupies the greater part of the sac , the embryonal area being situated opposite the intercotyledonary groove of the maternal placenta. As yet there is no organic connection between foetal and maternal tissues.

On the 8th day , as shown by Chipman , the foetal ectoderm first comes in contact with the maternal placenta. The latter has meanwhile advanced in development , so that the perivascular sheaths of decidual cells are developed round the vessels nearer the surface , while the covering epithelium has become further thickened and degenerated so as to constitute a thick plasmodial covering to the cotyledons. Over the lateral area of each cotyledon the foetal ectoderm , which is in one or two layers comes in contact with this plasmodial

tissue , and proliferates and penetrates into it. As the embryo is opposite the intercotyledonary groove , and the contact of the ectoderm is first made on the lateral areas of each cotyledon , it follows that this area of contact is situated some distance on each side of the embryo.

From this time onwards the foetal ectoderm continues to proliferate so as to extend its superficial area of contact , and also its depth , so that by the 9th day it covers a great part of each cotyledon - it is never in contact with the maternal structures in the intercotyledonary groove - and has penetrated and absorbed the surface epithelium so as to come into contact with the connective tissue of the maternal placenta. The foetal ectoderm at first extends along the obliterated glands , but later it finds its way along the sides of the superficial maternal capillaries , so that the line of contact of foetal and maternal structures becomes very irregular. On the free face of the foetal ectoderm there is a thin layer of mesoderm.

In the maternal placenta the perivascular decidual sheaths have been increasing in thickness , so that in the deeper parts they impinge on each other , and none of the original corium is visible. The capillaries in the deeper parts have also become dilated into sinus-like spaces , and the endothelium shows a tendency to swell. Nearer the foetal surface the

" intermediary region " becomes differentiated. The ground substance of the corium becomes oedematous and the seat of lymph deposit , while the perivascular sheaths are only one or two cells thick. As the foetal ectoderm encroaches on this area along the maternal vessels multinucleated cells develop. They are large vesicular cells with three or four nuclei , and are derived , as Chipman has shown , from the uninucleated decidual cells. They are found round the vessel walls , and in the oedematous tissue of this region. The intermediary region is marked off from the rest of the maternal placenta - the region of the uterine sinuses - by a line of multinucleated masses of protoplasm , which are the remains of uterine glands.

By the 10th day the allantois has reached the mesoderm covering the face of the foetal placenta , and the allantoic circulation is established. The mesoderm , carrying with it the allantoic vessels , now begins to grow into the foetal ectoderm , thus breaking it up into columns. At the same time the ectoderm towards the maternal placenta is penetrating more deeply , extending along , and replacing the swollen endothelium of the maternal vessels , and surrounding the blood cavities which are formed by the breaking down of the vessel walls. Besides the blood spaces there are small fibrinous deposits in the intermediary region , the result of

slow leakage from the vessels. The region of the uterine sinuses is entirely made up of the perivascular decidual sheaths , which have almost entirely replaced the original corium.

By the 12th day the foetal ectoderm has become more definitely broken up into columns by the invading mesoderm , while in the deeper parts , where it is invading the maternal tissues it has become plasmodial , individual cell outlines being lost. The structures separating the maternal from the foetal blood at this stage are one or two layers of ectoderm , some mesoblastic tissue , and the foetal capillary wall. The fibrinous deposit in the intermediary region is extending into the region of the uterine sinuses , and the decidual cells round the dilating sinuses are being converted into multinucleated ones.

From the 12th to the 26th day the foetal placenta undergoes a process of elaboration , whereby the original columns become divided up into finer and finer tubes by the ingrowth of mesodermic processes carrying blood vessels. At the same time it invades more and more deeply the maternal placenta , increasing in depth at the expense of the latter. The whole of the ectoderm becomes plasmodial , and by reason of the mesodermic invasion the thickness of tissue separating the two blood systems becomes less and less , until at the

20th day there is only a thin plasmodial ectodermic wall , separating the foetal capillary from the maternal blood sinuses , and even this thin ectoderm is in places lost , so that over small areas the foetal capillary is directly bathed in maternal blood. This is the condition of the foetal placenta up till parturition , which occurs at the 30th day.

While the foetal placenta is thus developing , the maternal is also undergoing important changes. The fibrinous deposit gradually advances into the region of the uterine sinuses from the intermediary region , and as it does so the uninucleated develop into multinucleated decidual cells , so that by the 16th day the dilated uterine sinuses with their sheaths of uninucleated decidual cells lie as islands in the fibrin tissue and multinucleated cells. The fibrin tissue however , does not extend so far as the attachment of the placenta to the uterine wall , so that in a zone next the musculature no multinucleate cells develop , and the sinuses remain quite small with only very slightly thickened endothelium. This is known as the zone of separation , and is slightly differentiated by the 16th day (fig. 13). The sinuses in the region of the uterine sinuses gradually increase in size , and approach more closely the face of the foetal placenta. By the 14th day their endothelial linings are greatly swollen , and there has been deposited round them

a layer of fibrin. This layer gradually increases in thickness , and ultimately comes to line the vessel , the swollen endothelial cells being cast off into the lumen of the vessels. This is the condition met with at the 16th to the 18th day (fig. 13).

By a gradual increase of this fibrin tissue , and by its extension out among the decidual cells these latter become compressed and atrophy and degenerate. There is in addition leakage through the fibrin vessel walls , which results in the deposit of coagulated lymph. This has embedded in it leucocytes , fibrin threads , and the chromatic debris of the decidual cells. By the 28th day the maternal placenta consists of sinuses lined by fibrin laminae , lying in a mass of coagulated lymph , containing fibrin threads , leucocytes , and the remains of the deicdual cells.

Parturition occurs on the 30th day , and the placenta separates through the zone of separation which has meanwhile become attenuated in structure owing to the shrinking of the decidual cells. There is never any fibrinous deposit in it , and the cells remain uninuclear throughout. (fig. 17).

So much by way of summing up the facts with regard to the development of the foetus and placenta in the rabbit.

METHOD OF CARRYING OUT THE EXPERIMENTS

METHOD OF CARRYING OUT THE EXPERIMENTS.

The stage of pregnancy was calculated from the date of insemination. Only in one case was a rabbit used which was already pregnant when brought to the laboratory. The stage of gestation was however easily arrived at by the study of the placenta of a sac removed at the operation.

The animal having arrived at the stage of gestation required it was anaesthetised with ether , and fixed down on its back to a holder. The abdomen was shaved and thoroughly cleansed with a solution of lysol. The instruments used were sterilised , and a fine piece of gauze wrung out of warm lysol solution was spread over the abdomen and limbs. A linear incision usually about 1½ inches long was made in the middle line between the mammae , and a short distance above the pubes , and the abdomen opened. The uterus , with its gestation sacs , usually at once presented itself , and was expressed from the abdomen with as little direct handling as possible. It was necessary to have all the sacs in view in this way in order to select for experiment and observation only those which were of equal size and presumably of the same stage of development , and also in order to be certain of the exact position in the horn of the sac punctured. A warm moist piece of gauze was placed over the extruded sacs.

One sac , or in some cases two , or more , were selected for puncture. The syringe used was an ordinary glass serum syringe , with a fine sharp needle. The needle was entered near the roof of the sac , and thus far away from the placenta , and great care was always taken to avoid injury to the foetus , either by the needle or by the fingers which steadied the sac. The fluid was then aspirated from the uterus. In some cases great suction was necessary , and in others not so much , the quantity of fluid obtained varying with the stage of gestation , as detailed under the several experiments. The sac became collapsed , and after withdrawal of the needle a little leakage occurred in some cases.

In the earlier experiments which I carried out this was all that I did , but later , owing to the necessity of obtaining a standard by which to judge of the results of the experiments , I in every case excised one of the normal sacs completely , always taking care to select one of the same size as those aspirated. I did not do this in the earlier experiments for fear of causing abortion. When I found that this did not follow the simple operation , with the necessary handling of the uterus during it , I was encouraged to excision of a " control " sac. The results have been entirely satisfactory. The only animal which aborted during the whole course of the investigation was the first one I operated on ,

but it was almost at full time at the date of operation. It is a remarkable fact that the rabbit's uterus tolerates such interference , a point which will be discussed later.

The method of excising the sac was a very simple one. A double ligature was passed through the mesometrium opposite the centre , and this was tied on each side between the sac to be removed and that on either side. The ligature was then passed completely round the pedicle , just as is often done in the operation of salpingo-oophorectomy , and the sac cut away above it. The uterus was then returned to the abdomen , and the abdominal wall closed with through-and - through horse hair sutures.

The animals as a rule made a perfect recovery. In only one case was there any sign of sepsis , there being a pocket of pus round the uterus. This case was of course rejected , and does not appear among the experiments which follow.

After the lapse of a certain number of days varying from 3 up to 14 in the different experiments the animal was killed by the administration of chloroform. The abdomen was immediately opened and the uterus excised completely , the vagina and mesometrium being previously ligatured so as to prevent the escape of blood from the placentae.

The uterus was then put into a 5% solution of formalin for from 24 to 48 hours. After being thus sufficiently

hardened the different sacs were cut across transversely , as nearly as possible in the centre. In some cases the roof of the sac was dissected off to show the relations of the foetus and membranes (figs. 5 and 10). The naked eye appearances , normal and abnormal , were noted , and then the sacs were again put into 5% formalin for 24 hours , and thereafter dehydrated in increasing strengths of alcohol , beginning at 50 % and finishing up with absolute alcohol. From the latter the tissues were transferred to cedar oil for 24 hours , and then put into the oven in soft paraffin , where they were allowed to remain for 12 hours before being embedded in hard paraffin. Serial sections were cut from the blocks , and the stains used in every case have been haematoxylin and eosine , the clearing medium being clove oil. An attempt to stain with Weigert's Fibrin stain was made , but was found to be unsatisfactory. The fixing and hardening of the placental tissues is comparatively easy , but considerable difficulty was experienced in getting sections showing the exact relations of the membranes , as the amnion especially becomes shrivelled up and distorted by the fixing and hardening agents. I know of no method by which this can be prevented. It has added considerably to the difficulty of determining exactly the arrangement of the membranes at the different periods of gestation at which the various experi-

ments have been performed.

The earliest period at which I have operated has been the 10th day , and at this time the fluid which is asporated is the yolk sac fluid , the amnion only having just become closed in , and containing very little fluid (fig 1). At the 12th day , at which time experiments II and VIII were performed , it is also the yolk sac which is aspirated , but in all the other experiments it has been the liquor amnii which has been withdrawn (fig 2).

I now proceed to describe in detail the several experiments. The method of description is as follows. A short account of the operation is given , and the condition of the abdomen and of the uterus as a whole at the subsequent post mortem described. The naked eye appearances of the sacs in transverse section is detailed , first the sac removed at the time of operation , then a normal sac removed post mortem , and finally the sac or sacs which had been aspirated. Thereafter the microscopic appearances are described in the same order. At the end of each experiment a short summary of the results obtained is given.

The latter part of the paper is taken up with a discussion of the general results of the whole series of experiments and with the conclusions to be drawn therefrom.

DETAILED ACCOUNT OF EXPERIMENTS

EXPERIMENT I

Operation when 16 days pregnant.

Animal killed when 19 days pregnant.

Interval = 3 days.

Operation :- On opening the abdomen there were found to be four gestation sacs in the left horn , and five in the right. The terminal tubal sac of the left horn was punctured , and 1.5 cc. of clear fluid withdrawn. The two terminal sacs of the right horn were similarly treated , and the same quantity of clear fluid got from each. The vaginal sac of this horn was excised for comparison , and before closing the abdomen the sac in the left horn which had been punctured was excised , the interval since its puncture being ten minutes.

The animal recovered perfectly from the operation , and was killed 3 days later , and the uterus excised. All the other abdominal organs were healthy. There was no peritonitis. In the left horn of the uterus were three well-formed normal sacs with abundance of fluid , and a scar marking the site of excision of the terminal one. In the right horn was one normal sac , similar in size to those of the left horn , while at the vaginal end was the scar of the excised

sac. The two terminal sacs which had been aspirated were much smaller than the others , and were firm to the touch , apparently containing very little fluid (fig. 3). There was a little lymph over the site of puncture of the more proximal of the two sacs.

DETAILED DESCRIPTION OF THE SACS.

1. Naked eye Appearances.

Normal Sac from Right Horn - excised at operation.

The sac (fig. 2) is ovoid in form , measuring 2.4 cm. in vertical by 2.1 cm. in transverse diameter. The placenta is 1 cm in thickness , the maternal and foetal portions being of equal depth. The space between the roof of the sac and the surface of the placenta is 1.5 cm. The foetal placenta is vascular and succulent looking , while the maternal is paler with a few more vascular areas. No zone of separation is differentiated. The foetus measures 1.3 cm. in length. The digits and limbs are formed.

Punctured Sac from Left Horn- removed 10 minutes after puncture

The sac is rounder in shape than the preceding , measuring 2.1 cm. in each diameter. The placenta is 1.1 cm. in depth , .6 cm. being maternal and .5 cm. foetal. The space between the placenta and roof of sac is 1 cm. There has thus ensued in this sac , within 10

minutes of the withdrawal of the fluid , a diminution in the vertical diameter. This is due to the contraction of the muscular coats; as compared with the normal they are markedly thickened , both in the placental and non-placental area. While the maternal placenta is equally as vascular as that of the normal sac , the foetal is distinctly less so. The whole placenta , as a result of the contraction of the uterine walls , is compressed from side to side , the foetal lobes being approximated to each other , so that the placenta looks thicker and more compact than the normal. There are no haemorrhages visible anywhere. The foetus has the same appearances as that of the normal sac.

Normal Sac from Right Horn - removed post mortem.

The sac is ovoid in shape measuring 2.8 cm. in vertical , and 2.5 cm. in transverse diameter. The placenta is 1 cm in depth - .4 cm. maternal , and .5 cm. foetal - while the space between it and the roof of the sac is 1.8 cm. The foetal placenta is very vascular , and stands out in marked contrast to the paler maternal portion. In the latter there is no zone of separation distinctly marked off (fig. 4,A). The foetus is 3 cm. in length. The limbs are perfectly formed and the abdomen closed.

Distal Punctured Sac of Right Horn - removed post mortem.

This measures 2.3 cm. in each diameter.

The uterine wall is very much thickened , both in the placental and non-placental area , measuring 2 cm. in contrast with .5 mm. in the normal. The placenta is 1.4 cm. in depth , and occupies a very much greater relative area of the sac than does the normal , while at the same time it is absolutely greater in depth. This is due to the compression of the placenta from side to side by the thickened uterine wall (fig. 4,B). The appearance of the placenta is very different from that of the normal. The distinction between the maternal and foetal portions is not so marked , owing to the diminished vascularity of the latter. The zone of separation in the maternal portion is very distinct in contrast with the normal. The whole placenta is dense and compact looking. There are no haemorrhages. The space between the placenta and the roof of the sac is .9 cm. in contrast with 1.8 cm. in the normal sac. The foetus is only 1.3 cm. in length - the same size as the foetus in the sac excised at the operation, and very much smaller than that from the normal sac removed post mortem (fig. 5). It differs in appearance from both. While the normal foetus from the sac excised at the operation is of rounded contour , and of a semi-translucent colour , that from the punctured sac is flattened from side to side , is of a dull grey , opaque colour , and has obviously been dead for some time - probably from the time of operation as

development is equally advanced in the two (fig.5,D).

Proximal Punctured Sac of Right Horn - removed post mortem.

This sac , which in external appearance exactly resembled the preceding , was dissected with the object of showing the relations of the foetus and membranes. The wall of the uterus was dissected off the latter , and the same was done with one of the normal sacs from the left horn , and the two are contrasted in fig. 5. In the punctured sac the foetus is seen lying on the surface of the placenta. It is compressed from side to side , and enclosed in its amniotic sac , which contains only a few drops of fluid. The contrast between the size of the two sacs is very striking , as is also the difference in colour of the two foetuses - the normal one pink and translucent , the abnormal dull grey and opaque.

2. Microscopic Examination.

Normal Sac from Right Horn - excised at operation.

The wall of the sac is 4 mm. in thickness. The two muscular coats are readily distinguishable , as there is a good deal of loose cellular tissue between them , while the bundles of fibres in each coat are similarly separated. The mucous membrane is flattened , all trace of the original villous processes being lost. Giant cells are

numerous in the non-placental area , situated in the submucosa, and in the inner muscular layer. They show all stages of degeneration.

Maternal Placenta :- No zone of separation is yet differentiated (fig. 13). The region of the uterine sinuses is entirely made up of uninucleated decidual cells with only a few strands of the original corium remaining , so that the arrangement of the former as special perivascular sheaths is lost (fig. 13). The sinuses are irregularly dilated spaces containing maternal blood , and lined with endothelium , which in many cases , especially towards the foetal surface , is distinctly swollen , and is being thrown off into the lumina of the vessels. In the intermediary region this is specially well marked , and here the decidual cells are polynuclear , with a small quantity of fibrinous deposit between them. The remains of the glands still persist as multinucleated masses of protoplasm.

Foetal Placenta :- This consists of fine columns of ectoderm divided up by the mesoderm carrying the foetal vessels. The ectoderm every where stains well , the nuclei standing out distinctly , although the protoplasm has become plasmodial in the deeper parts. The mesoderm is an exceedingly fine tissue with a few branching cells and extremely loose stroma. The foetal vessels are lined with a single layer of endothelium,

and contains nucleated foetal blood. At the junction of foetal and maternal portions the ectoderm is seen actively proliferating along the walls of the maternal sinuses , and islands of multinucleated decidual cells are here and there included in it.

Foetus :- The embryo is everywhere invested by a single layer of low cubical epithelium , each cell with a single round , darkly staining nucleus , centrally placed. The connective tissue is of a loose myxomatous character , the ground substance being exceedingly fine , and the cells few in number. They are spindle shaped and have branching processes; the nuclei are oval and are situated in the centre of the cells. Cartilage is just beginning to make its appearance in those situations where it is normally present. Fig. 29 shows the appearance of the connective tissue and the epithelial investment.

The heart is well developed with thick muscular walls and many trabeculae. The muscle cells are beginning to show striation , and dark uniformly staining nuclei are situated at regular intervals. The individual cell outlines are quite distinct (fig. 31). The cavity of the organ is lined by endothelium.

The liver (fig. 35) is large in size compared with the size of the foetus. It is made up of a loose net-work

of columns or tubules of large cubical cells , each with a centrally placed darkly staining nucleus. The connective tissue between the liver cells is very fine , containing few branched connective tissue cells. In it run many blood vessels which are filled with nucleated red blood corpuscles.

The kidney (fig. 39) has a capsule formed by the condensation of the connective tissue at the margin of the organ. The tubules are already formed and are wide , with a lining of cubical epithelium in a single layer , each cell having a round , deeply placed nucleus which stains uniformly. The glomeruli are numerous , consisting of a fine capsule , and a vascular tuft in which are many nucleated red blood corpuscles.

The pancreas is visible as an outgrowth behind the stomach , and consists of a loose stroma , in which are embedded branching glands lined by a cubical epithelium. The stomach has thick walls , and is lined by a double layer of tall columnar epithelial cells.

Punctured Sac from Left Horn - removed at operation 10 minutes after puncture.

The only differences between this and the preceding are (1) the greater thickness of the wall of the sac - .7 mm. as compared with .4 mm - this thickening being due to the contraction of the muscular coats; (2) the

compression of the foetal placenta , so that the columns are not so open as in the normal sac. This latter is due to the contraction of the muscular coat. There are no haemorrhages either in the foetal or in the maternal placenta. The foetus presents exactly the same appearances as the normal.

Normal Sac from Right Horn - removed post mortem.

The wall is .8 mm. in thickness. The mucosa is flattened , the epithelium lining the cavity , and the glands show no tendency to proliferate. A few degenerated giant cells are present.

Maternal Placenta :- The zone of separation is just beginning to be differentiated as an area consisting of uninucleated decidual cells and a few small uterine sinuses. The rest of the maternal portion is made up of large maternal sinuses , some of which still retain their swollen endothelial lining , while in others this has been replaced by fibrin laminae. The sinuses are surrounded by decidual cells , between which fibrin threads and laminae extend in all directions. The intermediary zone as such has disappeared , owing to the invasion of the foetal placenta. There are large deposits of fibrin in the lateral portions.

Foetal Placenta :- This differs from that of the sac removed at the operation only in its greater depth , the finer subdivision of the ectodermic columns , and the preponderance

of the plasmodial ectoderm over the cellular. Everywhere the nuclei stain well. The mesoderm is a fine cellular tissue with no fibrinous deposit.

Distal Punctured Sac of Right Horn - removed post mortem.

The wall is thickened measuring 2 mm. both in the placental and non-placental areas. This thickening is in both the muscular and mucous coats. In the former both layers are compact , and there is no cellular layer between them. The fibres are evidently in active contraction. In the mucous coat there is proliferation of the sub-mucous tissue which is very cellular , and which is growing out as villous processes covered with epithelium. The epithelial lining everywhere is proliferating so that an appearance of numerous glands is produced. A few giant cells are present.

Maternal Placenta :- The zone of separation is more distinctly marked off than in the normal sac owing to a slightly looser arrangement of the cells , and to the fact that there is more fibrinous deposit in the rest of the placenta than normal which makes the contrast more striking. The rest of the maternal placenta has exactly the same structure as the normal 19 day sac , the only difference being the slightly greater amount of fibrin deposited between the decidual cells. It has advanced in development from the condition present at

the 16th day. There are no haemorrhages.

Foetal Placenta :- The most striking feature in this is the density of the mesodermic tissue at the surface of the placenta and in its deeper parts. It is more compact and cellular than normal , the cells being spindle shaped with long processes , and in addition there is an extensive deposit of fibrin throughout (fig. 30). Vessels are scanty , and where traced have usually their walls in apposition and contain no blood. The ectoderm shows very little change from normal. In one or two isolated , irregularly distributed areas the nuclear staining is faint or altogether lost , but for the most part there is no degeneration. At the junction with the maternal placenta the ectoderm is seen proliferating along the walls of the maternal vessels just as in the normal 19 days sac.

Foetus :- The surface epithelium has entirely disappeared. The cellular tissue has lost its myxomatous appearance , this being due to the closer aggregation of the cells , and to an alteration in their character. Instead of being spindle shaped they appear round , owing to the nuclei having become swollen and rounded; many of them also are granular and evidently in a state of degeneration (fig. 30). There is only a faint indication of cartilage formation , such as is seen in the normal 16 days foetus , and in contrast to the

19 days one where cartilage is fully formed.

The heart shows very marked evidences of degeneration (fig. 32). The fibres are swollen , many of them are split longitudinally , the protoplasm is granular , and the cell outlines indistinct. The nuclei stain faintly , and many of them are granular. The endothelial lining is lost in places.

The liver (fig. 36) is denser than normal , the columns of cells being closer. The outlines of the liver cells are in many cases not visible , and the protoplasm stains darkly but irregularly with eosine. Many of the nuclei are swollen , so that their edges are indistinct , and are in a state of granular degeneration. Between the columns of liver cells the connective tissue cells are more numerous than normal , and they have undergone the same change as that described in the connective tissue elsewhere. In it there are many broken-down blood corpuscles and debris. Most of the corpuscles are no longer round , and the nuclei stain faintly. All these changes are well shown in fig. 36.

The kidney (fig. 40) shows the same stage of development as that of the 16 days embryo. The epithelial lining of the tubules is intact , but the cells are swollen , and there is a tendency for the protoplasm at their free ends to break down. The nuclei stain faintly , but are still

situated in the deep part of the cell. The general appearance of the cells is like that usually described as cloudy swelling. In the glomeruli the capsule comes close up to the tuft , and the latter is compressed although nucleated foetal blood is still present in it. The connective tissue between the tubules and glomeruli is more abundant than normal, and the cells are of the same character as in the liver.

There is only slight departure from normal in the epithelium of the pancreatic glands - it is slightly swollen. The connective tissue is dense , and the cells are rounded with swollen granular nuclei.

SUMMARY OF RESULTS OF EXPERIMENT I.

From the examination of the sac which was aspirated at the beginning and excised at the end of the operation , it would appear that the first effect of the withdrawal of the fluid is to set up contractions in the muscular wall of the uterus , as evidenced by its marked thickening. This muscular contraction causes compression of the placenta from side to side , bringing the two foetal lobes into closer approximation , and it also counteracts to some extent the negative pressure in the sac which must necessarily result when the contents are withdrawn and no air enters. This latter action is doubtless an important one in determining the changes which subsequently take place in the placenta , especially in the prevention of haemorrhage into it , and into the sac. As was noted , no haemorrhages were anywhere visible.

From the examination of the punctured sacs examined 3 days after the operation it is evident that there has been no re-secretion of fluid - what little there was , was no more than was left at the time of operation. Comparing the foetuses from the punctured sacs with that of the normal sac removed at the operation it is evident that they are equally advanced in development. The foetuses in the punctured sacs also show very marked degenerative changes , both naked eye and microscopically , so that the probability is that they

have died very soon after the operation. There was no evidence of any injury to them and great care was taken at the operation to avoid such , so that we must conclude that their death has resulted from the withdrawal of the fluid , but in what way this has acted there is no direct evidence. The flattening of the foetuses from side to side between the face of the placenta and the roof of the sac is a noteworthy feature.

The thickening of the muscular coats of the punctured sacs is very marked , but the changes in the placenta are slight. There is more marked compression from side to side and approximation of the two lobes in the middle line than was noted in the sac removed 10 minutes after puncture. The maternal placenta in both sacs has gone on developing along the normal lines from the time of the operation to the death of the animal , the only departure from normal being an increased deposit of fibrin , and this is rather in the direction of accelerated than of retarded growth. The changes in the foetal placenta are the fibrinous deposit in , and condensation of the mesoderm, the slight compression from side to side of the ectodermic columns and consequent compression of foetal vessels. The ectoderm shows least departure from normal , a want of nuclear staining being evident only in small scattered areas. The comparatively normal

appearance of the placenta is in very marked contrast to the pronounced changes of the foetus.

EXPERIMENT II.

Operation when 12 days pregnant.

Animal killed when 17 days pregnant.

Interval = 5 days.

Operation :- On opening the abdomen there were found to be two gestation sacs in the right horn and four in the left , all being of the same size. The two right horn sacs were aspirated , and ½ cc. of turbid fluid withdrawn from each , so that both were left quite flaccid. One of the sacs in the left horn was excised completely.

The animal recovered perfectly from the operation , and was killed 5 days later when the following conditions were found.

The right horn sacs were very much smaller than those of the left , and they contained no fluid - had very much the same appearance they had at the end of the operation (fig. 6). The sacs in the left horn had increased very considerably in size , and contained a large quantity of fluid. There were no changes in any of the other abdominal or pelvic viscera.

DETAILED DESCRIPTION OF THE SACS.

1. Naked eye Appearances.

Normal Sac from Left Horn - excised at Operation.

On section it is almost circular in

outline , measuring 2 cm. in the vertical by 2 cm. in the transverse diameter. The placenta presents the ordinary appearances of a 12 days pregnancy , the foetal part being readily distinguished from the maternal by its dark red colour. In the maternal part no zone of separation is yet visible. The whole thickness of the placenta is 8 mm. , the maternal and foetal portions being of equal depth. The space in which the foetus lies - between the surface of the placenta and the roof of the sac - is 1.2 cm. The foetus measures 1.5 cm in length.

Normal Sac from Left Horn - removed post mortem.

On section it is oval in outline , measuring 3 cm. in the vertical and 2.4 cm. in the transverse diameter. The placenta is 1.1 cm. in depth , the foetal and maternal parts being each .55 mm. , and the distinction between the two being very evident because of the contrast in vascularity. The zone of separation in the maternal placenta is faintly visible as a whitish line next the wall of the sac. The space in which the foetus lies is 1.5 cm. in vertical measurement. The foetus measures 2.5 cm. in length , and is perfectly formed.

Distal Punctured Sac from Right Horn - removed post mortem.

On section it is almost circular in outline , measuring 2.1 cm. in vertical , and 2 cm. in trans-

verse diameter - almost exactly the same as the 12 days sac. The wall of the sac is thicker than that of either the 12 or the 17 days normal one. The most striking feature is the relatively large area of the sac which is occupied by the placenta , the thickness of which is 1.3 cm. The distinction between the maternal and foetal placenta is not nearly so well marked as in the normal 17th day sac , this being due to the loss of vascularity in the foetal portion. In the maternal placenta the zone of separation is very distinctly seen - much more so than in the normal sac. There is no evidence of any haemorrhage into the placenta. The space between the surface of the placenta and roof of the sac measures only 6 mm. , as compared with 1.5 cm. in the normal sac. This space is occupied by a dark semi-gelatinous substance , and the remains of the foetus are visible in it as a small , flattened , grey body , measuring only 8 mm x 1 mm.

Proximal Punctured Sac from Right Horn - removed post mortem.

Measurements :- 2.1 cm. in vertical and 2.1 in transverse diameter. The general appearance of this sac is very much the same as that of the preceding. The placenta is 1.4 cm. in thickness , and while the distinction between the foetal and maternal parts is quite evident (the former being more vascular than in the distal sac) it is very much less so than in the normal. There is no

evidence of any haemorrhage. The space between the face of the placenta and the roof of the sac is only 8 mm. , and the foetus is again represented by a small grey body measuring 9 mm. x 2 mm. It has evidently been dead for some time.

2. Microscopic Examination.

Normal Sac from Left Horn - excised at operation.

The wall of the sac is thin , .5 mm. The distinction between the two muscular coats is very well marked with large dilated vessels filled with blood between them , these as usual being more abundant at the placental site. The mucosa is for the most part flattened , but at one or two places is raised into cushion-like areas by the proliferation of the sub-mucous tissue and formation of decidual cells. It is lined by a single layer of columnar epithelium. A few villous processes are present. In the non-placental part giant cells are numerous presenting various stages of degeneration , and in places invading the inner muscular wall.

Maternal Placenta :- No zone of separation is as yet differentiated.

Region of the Uterine Sinuses :- The great bulk of the tissue is made up of uninucleated decidual cells , only a few strands of the original corium being present. The sinuses

are dilated irrgularly and filled with maternal blood. The endothelial lining is swollen in all the larger ones , and it is only round the smaller that the decidual cells can be defined as forming a definite perivascular sheath. This region makes up the greater part of the maternal placenta , being four times the thickness of the intermediary region.

Intermediary Region :- As this region is approached the uterine sinuses become larger , and the epithelium more swollen, while the uninucleated decidual cells are more loosely arranged. In the region proper there is an almost continuous band of fibrin deposit which limits it from the reion of the uterine sinuses. In the deposit are , here and there , small islands of multinucleated protoplasm - the remains of uterine glands. Towards the foetal surface the decidual cells are multinucleated and very loosely arranged round the maternal vessels , which in most cases have still their endothelial lining. The foetal ectoderm at its areas of contact is invading this region , and extending along the maternal vessels , cutting off at places islands of multinucleated decidual cells. In the lateral regions of the placenta - beyond the line of contact with the foetal ectoderm - there is a good deal of fibrinous deposit , especially in the superficial part.

Foetal Placenta :- This consists of the foetal ectoderm

broken up into columns by the mesoderm carrying with it foetal vessels. In the deeper parts the ectoderm is seen extending along the walls of the maternal vessels. In the central parts the ectoderm is assuming a plasmodial character , but everywhere the tissue seems to be active , both cell body and nucleus readily taking up their distinctive stains , and showing no signs of degeneration.

Foetus :- The surface is covered with low cubical epithelium, each cell with a centrally placed , darkly staining nucleus. The connective tissue is of loose myxomatous character with spindle-shaped cells , set far apart. In the regions where the vertebrae will develop the connective tissue cells are rounder and set closer together , but as yet have not taken on the character of cartilage cells.

The heart has thick walls with numerous trabeculae crossing the cavity. The muscle cells do not yet show any striation , their individual outlines are quite distinct , and the nuclei are large and round and stain deeply. The endothelial lining is intact.

The liver is an open net-work of round and cubical cells arranged in columns. The cell outlines are well marked , and each has a large round nucleus. There is much blood in the loose connective tissue which separates the liver cells proper.

The kidney is covered with a layer of cubical cells. The glomeruli are large and fully formed with a capsule and tuft. They contain much nucleated blood. The tubules are wide and are lined with cubical epithelium , with deeply set , darkly staining nuclei. The connective tissue is loose and vascular.

The pancreas is just beginning to develop as a glandular organ behind the stomach.

Normal Sac from Left Horn - 17 days pregnant.

The wall presents very much the same appearances as that of the 12 days sac (fig. 14). The mucosa is flattened , and the sub-mucous tissue rather abundant. Giant cells are still present although they do not invade the inner muscular wall to the same extent. They also show a more advanced stage of degeneration , in many of them no nuclei being visible , and in others the nuclear staining being very faint.

Maternal Placenta :- The chief changes from the preceding are :- the large size of the uterine sinuses and the loss , to a great extent , of their endothelial lining , which is replaced by thin laminae of fibrin :- the beginning differentiation of a zone of separation which is composed of large vesicular uninucleated decidual cells , and contains no large vessels. There is an increased deposit of fibrin towards

the foetal part of the intermediary region. The fibrin is invaded by uninucleated leucocytes , multinucleated giant cells , while lying in it the remains of decidual cells are visible. Towards the foetal placenta the tissue is very loose , and there are large blood spaces due to the breaking down of the vessel walls , and along these walls the foetal ectoderm is extending , so as to enclose the maternal blood spaces.

Foetal Placenta :- It is very much as it was at the 12th day , but is broken up into finer columns by the mesoderm. The ectoderm is now in great part plasmodial , but everywhere stains well. The foetal vessels are seen running along in the mesoderm , separated from the maternal vessels by the ectoderm and the endothelial lining. At the maternal junction the ectoderm is actively proliferating along the sinus walls (fig. 1).

Foetus :- This has the same structure as that described in the 16 days foetus in experiment I.

Distal Punctured Sac of Right Horn - removed post mortem.

The muscular wall at the placental site is 1.2 mm. in thickness , while in the 12th day sac it is .5 mm. , and in the normal 17th day sac .75 mm. (figs. 14 and 15). The muscular fibres are rather loosely arranged , and there are large clear areas between them. There are a

few large vessels between the coats , the distinction between which is quite distinct. The mucosa is here and there raised into thick , cushion-like areas by the development of the sub-mucous tissue , and formation of decidual cells. These areas are invaded by giant cells which are present in large numbers and in all stages of degeneration throughout the non-placental part of the mucosa and inner muscular wall. They are more numerous than in the normal sac. The lining epithelium and glands show here and there a tendency to proliferation.

Maternal Placenta :- The zone of separation is more distinctly marked than in the normal 17th day sac , and differs from it further in the general more loose arrangement of the tissue due to the shrinkage of the decidual cells (figs. 14 and 15). The appearances differ from the 12th day sac in which no zone of separation is marked off.

Region of the Uterine Sinuses :- At the 12th day the characteristic features were the large sinuses with swollen endothelium , and the large number of uninucleated decidual cells. At the 17th day we noted the dilated vessels with absence of endothelial lining and the substitution of laminae of fibrin. In the sac under consideration the changes have gone on , to a certain extent , in the normal manner. Thus the endothelial lining of the vessels has been in great part lost and re-

placed by fibrin , and their shape has altered from the irregular contour seen at the 12th day to the longitudinally running tubes which are normal at the 17th. There is however , more fibrinous deposit among the decidual cells than normal at this period of gestation , and in the lateral parts of the placenta there are large areas composed entirely of fibrin tissue and thrombosed vessels. Uninucleated and multinucleated giant cells , together with red blood corpuscles and leucocytes are present in the fibrinous deposit. Many of the multinucleated decidual cells are shrunken , and some have lost their nuclei.

With these exceptions there is then no difference between this placenta and the normal 17th day one.

Foetal Placenta :- This is smaller in extent than normal at the 17th day. The tissue is dense and solid looking in contrast to the normal. This is in great part due to the condensation of the mesodermic tissue which in the normal sac is an abundant , loose , cellular tissue containing foetal vessels and dividing the ectoderm into columns. In the sac at present under consideration this division into columns is hardly distinguishable (fig. 25). At the surface of the placenta the mesoderm is denser than normal , and throughout there are fine bands of fibrin which here and there are pressed together so as to form fairly thick laminae (fig 25).

The vessels in it are few in number. The ectoderm has also undergone very considerable changes of a degenerative character , so that on low power view it has a more or less homogeneous purple appearance owing to the deficient nuclear staining. This degeneration is most marked towards the centre and least so towards the maternal placenta. In the central area many of the nuclei have entirely disappeared and the protoplasm of the cell bodies is also undergoing degeneration , taking on an irregular purplish staining with haematoxylin and eosine , no cell outlines being distinctly visible. A few maternal vessels are visible , but the foetal ones have been , for the most part , obliterated by the pressure. It must be remembered that at this stage the foetal vessels are not easily distinguished as the blood is ceasing to be nucleated. Towards the foetal surface these degenerative changes are not so marked. The ectoderm is more reticulated and broken up , and here and there cell outlines can be quite distinctly seen , while the nuclei have not disappeared to nearly the same extent. Both maternal and foetal vessels can be seen. Towards the maternal placenta the changes are still less marked as regards the cellular degeneration - cell outlines are quite distinct but the nuclear staining is fainter than normal , and in some cases absent , while many of the cell bodies stain irregularly. The most striking difference

between this region and the corresponding one of the normal sac is the absence of active proliferation and extension of the ectoderm into the maternal placenta along the blood spaces. This was a very noticeable feature both in the 12th and in the 17th days sacs , but here it is almost entirely absent (figs. 18 and 19).

Foetus :- There is no epithelial covering over any part. The connective tissue is entirely different from normal (fig. 30). The ground substance is a granular matrix , resulting from the breaking down of cell protoplasm , and the exudation of lymph , and in this the cells lie much more closely arranged than normal. Most of the cells are altered in outline , and many of them are irregular in shape. The nuclei of the cells are swollen , with outline blurred , and containing in their interior many fine granules. There is no attempt at cartilage formation.

The heart shows very extensive degenerative changes. The fibres are granular and stain faintly and irregularly , and the cell outlines are blurred. In some cases the nuclei have entirely disappeared , in others they still take on the stain , but that irregularly , so that they appear granular. The endothelium is everywhere raised off the surface , and is being shed into the cavity (fig. 33). In the interior of the cavity the red corpuscles are collected into groups.

They have irregular outlines , and the nuclei stain faintly and irregularly. Fine strands of fibrin are also present.

The liver is much broken up (fig. 37). The liver cells are aggregated into groups in which no distinct cell outlines can be distinguished, The protoplasm stains deeply , but irregularly , with eosine , while the nuclei are swollen and show very marked granular degeneration.- much more than in the preceding experiment. The connective tissue between the liver cells is loose , and in it are the remains of broken down blood corpuscles and other debris , while the cells have large swollen granular nuclei.

The kidneys show changes very similar to those observed in the preceding experiment. The glomeruli are shrunken , and contain only a few degenerated red corpuscles. The epithelium of the tubules is slightly swollen and stains irregularly. Some of the nuclei are faintly granular. The changes in the renal epithelium are not nearly so marked as those in the liver cells. The connective tissue cells are numerous and are swollen and rounded with granular nuclei.

The epithelial cells of the pancreas are swollen and the nuclear staining is faint.

Membranes :- The yolk sac wall has in places lost its epithelial covering. Where present there is swelling of the cells , granulation of the nuclei , and a tendency to the loss

of individual cell outlines and the formation of a plasmodium. The mesoderm contains fibrin and extravasated lymph in which are the remains of degenerated blood corpuscles.

The amnion shows no departure from normal.

The chief changes in the sac then are :-

1. Thickening of the muscular coat.
2. Proliferation of sub-mucous tissue , and surface and glandular epithelium.
3. Increased deposit of fibrin in the maternal placenta.
4. Compression of the foetal placenta.
5. Condensation of the foetal mesoderm in the foetal placenta , and the deposit in it of strands of fibrin.
6. Degeneration of the foetal ectoderm and the loss of its power of penetration.
7. Degeneration of the foetus.

Proximal Punctured Sac from Right Horn - removed post mortem.

Wall. Thickness at placental site = 1.75 mm. As in the other punctured sac the muscular fibres are loosely arranged with many clear intervals between them. The distinction between inner and outer muscular coats is

well marked , large vessels being here and there visible between them. The mucosa is thicker than in the normal sac , this thickening being chiefly due to an increase in the submucous tissue which here and there forms large cushions. In these areas decidual cells are present. There is also some proliferation of the surface and glandular epithelium. Giant cells are more numerous than in the normal sac , and show as a whole a more advanced degenerative change.

Maternal Placenta :- The zone of separation is distinct , and as in the preceding sac the tissue is looser than normal. The rest of the maternal placenta corresponds in appearance with that just described , differing from the normal in the more extensive fibrinous deposit and blood extravasation.

Foetal Placenta :- This is about the same size as that of the normal sac , but differs from it in being denser looking and more homogeneous. This latter appearance , as in the former case , is due to the degeneration of the foetal ectoderm with the consequent partial loss of nuclear staining. Here again the condensation of the mesoderm is noted , and also the fibrinous deposit in its surface layers. There is slightly more mesoderm present than in the previous sac , so that the division of the ectoderm into columns is visible in places , especially towards the foetal surface. The degeneration of the ectoderm is not quite so marked as in the other

punctured sac , but is still very evident: and as in it ,is most advanced in the central areas , and least so towards the maternal placenta. Thus in the central area cell outlines are almost completely lost , the protoplasm is granular and vacuolated , and the nucleus has in many cases disappeared , and where present shows irregular staining. Towards the foetal surface these changes are less marked. There are one or two large areas of haemorrhage , which have completely replaced the normal tissues. Foetal vessels are throughout very scanty. Towards the maternal surface , while the degenerative changes are less marked than elsewhere , the absence of active proliferation of the ectoderm , and extension along the walls of the maternal vessels is noteworthy.

The foetus and membranes show the same changes as those just described in the distal punctured sac.

This sac therefore , shows changes of exactly the same nature as the preceding , although these changes differ slightly in degree , viz. :-

1. The less advanced degeneration of the foetal ectoderm.
2. The greater abundance of foetal mesoderm.

SUMMARY OF RESULTS OF EXPERIMENT II.

The changes in the punctured sacs are of the same nature, but of a more advanced stage than , those noted in the preceding experiment. There has been no re-secretion of the fluid. The foetuses have died and are very extensively degenerated , and the probability is that they have been dead since the operation. The muscular walls of the uterus show the same marked hypertrophy. The changes in the maternal placenta are insignificant when contrasted with those which have occurred in the foetus , and as before these changes are rather in the direction of accelerated development than of any degenerative process. The results in the foetal portion of the placenta are very evident , and are a further advance on the degenerative changes noted in the preceding experiment. The interval of time between the aspiration of the sacs and the death of the foetus in this experiment was 5 days as compared with 3 days in experiment I , and the changes which have occurred in the 18 hours are quite evident in the larger area of the ectoderm which has degenerated , and the more advanced nature of that degeneration. But the difference is merely one of degree , the process being identical in the two cases.

EXPERIMENT III.

Operation when 14 days pregnant.

Animal killed when 21 days pregnant.

Interval = 7 days.

Operation :- On opening the abdomen there were found to be five gestation sacs in the right horn and one in the left. The latter and the two proximal sacs of the right horn were punctured , and the fluid withdrawn. The left horn sac was left quite flaccid , but all the fluid could not be withdrawn from the right horn , though the greater part was. One of the sacs in the right horn was excised completely. The animal made a good recovery and was killed after an interval of 7 days. In the right horn were two apparently normal sacs containing abundance of fluid , and separated from each other by the cicatrix of the excised sac. The two proximal sacs which had been punctured were very much smaller and contained practically no fluid. The left horn sac was very small and contained no fluid (fig. 7).

DETAILED DESCRIPTION OF THE SACS.

1. Naked eye Appearances.

Normal Sac from Right Horn - excised at operation.

This measures 2.3 cm. in the vertical

and 1.9 cm. in the transverse diameter (fig. 8,A). The placenta is 1 cm. in thickness. The foetal is quite distinct from the maternal portion , the former being red and vascular and of horse shoe shape. The foetal placenta is 5 mm. and the maternal 6 mm. in thickness. The foetus is 1.3 cm. in length. The space in which it lies is 1.3 cm in height.

Normal Sac from Right Horn - removed post mortem.

This measures 3.1 cm. in the vertical , and 2.8 cm. in transverse diameter (fig. 8,B). The depth of the placenta is 1 cm. - foetal 7 mm. and maternal 3 mm. The foetal placenta is large , vascular and succulent looking. The maternal placenta is pale with a few small haemorrhagic areas. The zone of separation is faintly visible. The foetus is 3.1 cm. in length. The height of the space in which it lies is 1.9 cm.

Punctured Sac from Left Horn - removed post mortem.

This is circular in outline , measuring 1.9 cm. in vertical , and 1.9 cm. in transverse diameter - less than the 14th day sac (fig. 8,C). The placenta is 1.3 cm. in depth , and thus almost completely fills up the sac , leaving a space of only 4 mm. between it and the roof. In this space the foetus , which is represented by some greyish debris , lies. The walls are very markedly thickened

especially at the area of placental attachment. The distinction between maternal and foetal placenta is not nearly so marked as in the normal sac. In the latter are many haemorrhagic areas , giving it a mottled appearance , while it looks more friable and less succulent than the corresponding area in the normal sac. The two lobes of the placenta have been pressed together , but the foetal parts still retain their horse shoe shape. In the maternal placenta the zone of separation is more clearly differentiated than in the normal 21 days placenta. There are very few haemorrhages in this part.

Proximal Punctured Sac from Right Horn - removed post mortem. Vertical diameter = 2.2 cm: transverse diameter = 1.4 cm. Placenta is 1.4 cm. in depth , maternal 1 cm. and foetal 4 mm. The space between the placenta and the roof of the sac is only 5 mm. so that here again the placenta occupies almost the whole of the sac. The uterine wall , as in the last sac , is very much thickened - 1.5 mm. at the placental site , and 1 mm. opposite it. The distinction between foetal and maternal placenta is well marked, but the extent and depth of the former is much less than normal, 4 mm. instead of 7 mm. It retains its horse shoe shape , and there are very few haemorrhages into it. In the maternal placenta the zone of separation is very well marked.

The foetus is very small , and is flattened out between the top of the placenta and roof of the sac - like a foetus paparaceous. It is of a reddish colour and has evidently been dead for some time.

Distal Punctured Sac from Right Horn - remomoved post mortem. Vertical diameter = 2.5 cm. Transverse diameter = 2.1 cm. Depth of placenta = 1.3 cm. Space above placenta = 2.1 cm. This sac therefore differs from the preceding in the large space between the placenta and the roof of the sac , but this is due to the fact that the foetus in its membranes belonging to the adjoining sac , which was a normal one , projected into it , the intervening constriction of the horn having been opened up. This free space above the placenta was thus occupied by the contents of another sac. It is probable that it was so opened up only after the death of the animal , as I have frequently noted the initial stages of parturition occur just on the death of the animal.

The appearance of the placenta is very much the same as in the other two. The foetal part is paler and smaller than normal - only 4 mm. in thickness. The zone of separation in the maternal portion is well marked.

Lying on the surface of the placenta and enclosed in its membranes are seen the remains of the foetus. It is only 6 mm. in length , and is flattened out on the surface of the

placenta. No limbs or other parts can be distinguished. There is no fluid in the membranes.

2. Microscopic Examination.

Normal Sac from Right Horn - excised at operation.

The wall of the sac is .8 mm. in thickness. The two muscular coats are separated by large vessels. The submucous tissue is scanty , but in two areas it is abundant , giving rise to cushion-like elevations. Giant cells are numerous. The surface of the mucosa is for the most part flat , but here and there are short villous processes. These are more numerous towards the placental area.

Maternal Placenta :- Region of Uterine Sinuses. The whole of this area is made up of uninucleated decidual cells. The sinuses are irregularly dilated spaces with swollen endothelial walls. There is no zone of separation.

Intermediary Region :- consists of polynuclear decidual cells surrounding irregularly shaped sinuses containing maternal blood , and lined by swollen endothelium or laminae of fibrin , which has in some cases replaced the endothelium. There is some fibrinous deposit towards the foetal surface , and here and there islands of multinucleated protoplasm are visible. At the line of contact of the foetal with the

maternal placenta the ectoderm of the former is extending along the walls of the uterine sinuses and blood spaces.

Foetal Placenta :- This consists of the foetal ectoderm broken up into many columns by the mesoderm - a very loose tissue with few cells , and carrying with it the foetal vessels which are everywhere visible filled with nucleated red blood corpuscles. The whole tissue has a loose appearance, especially at the foetal and maternal surfaces , the central part being denser. The ectoderm nowhere shows any tendency to degeneration although it is beginning to be plasmodial. Nuclear staining is everywhere distinct. At the junction with the maternal placenta there is active proliferation and extension along the vessels.

Foetus :- It is covered with a single layer of low epithelium , each cell with a round nucleus. The connective tissue is loose and myxomatous , with spindle-shaped , branching cells set at wide intervals in the scanty ground substance. There are numerous vessels in it. Cartilage has not yet been fully formed , although in the places where it is to develop aggregations of round cells are present.

The heart has thick walls composed of muscle fibres with deeply staining nuclei. The individual cell outlines are distinct but striation is not yet visible.

The liver consists of cubical and rounded cells with

central deeply staining nuclei arranged in a loose net-work , between the columns of which is loosely arranged connsctive tissue with branching spindle cells , and many blood vessels containing nucleated foetal blood.

The kidney has a capsule of low cubical cells. The glomeruli are large and the tufts are full of nucleated red blood corpuscles. The tubules are wide , and are lined by cubical epithelium , the protoplasm of which stains uniformly. The nuclei are situated deep in the cells and stain darkly. The connective tissue between the tubules and glomeruli is loose and open.

The pancreas is already developed behind the stomach , and consists of branching glands embedded in a loose stroma.

The stomach has thick walls and is lined by a double layer of tall columnar epithelium.

Normal Sac from Right Horn - removed post mortem.

The sac is ovoid in shape. The wall is .5 mm. in thickness. The muscular fibres are loosely arranged , especially those of the outer coat. Large vessels are numerous between the muscular coats. The mucosa is flattened , only a few glands being visible , and the sub-mucous tissue is scanty except in one place , where it has been transformed into decidual tissue , and forms a thick cushion on the lateral uterine wall. Giant cells

are few in number and do not invade the muscle wall.

Maternal Placenta :- The zone of separation , consisting of uninucleated decidual cells and a few small vessels is well marked. The rest of the placenta is small in extent , being of only about half the thickness of the foetal part. It consists of fibrinous deposit with various cells entangled in it and islands of multinucleated decidual cells , surrounding the fibrin-lined uterine sinuses. In some of the latter the swollen endothelium is still present on the inner surface of the fibrin laminae.

Foetal Placenta :- This is twice the thickness of the maternal. Nowhere does it show any tendency to degeneration. The mesoderm is everywhere a very loose tissue , especially abundant at the foetal surface , but scarcely visible in the deeper parts. There is no fibrinous deposit at any part. The ectoderm is broken up into very fine columns and shows no degenerative change - nuclear and protoplasmic staining are quite distinct. At the junction of foetal and maternal tissues there is active proliferation and extension along the maternal blood spaces (fig. 20). There are no areas of haemorrhage. The foetus is cut transversely through the liver: the cartilages and the limbs are also cut across. There is no degenerative change in it.

Punctured Sac from Left Horn - removed post mortem.

The wall at the placental site is 1.75 mm. in thickness , this thickening being due to contraction of the muscular coats which are dense and compact. At the placental attachment large vessels separate the two coats. The mucosa is thrown into many villous folds , these being especially large and numerous towards the placental site. The stroma of the mucous membrane is composed of a well developed tissue , no decidual cells being present. The glands and surface epithelium show a tendency to proliferation. Giant cells are numerous and large. They present all stages of degeneration , and in many places invade deeply the inner muscular coat.

Maternal Placenta :- The zone of separation is well marked , and consists of a loose stroma made up of branching decidual cells. The rest of the maternal placenta consists of extravasated blood and fibrin which are invaded by leucocytes , multinucleated giant cells and phagocytes. The maternal sinuses lie in this tissue as islands surrounded by sheaths of polynuclear decidual cells. The lining epithelium , where present , is swollen , but has for the most part been replaced by fibrin. Many of the vessels are thrombosed. The general structure of the placenta is the same as that of the normal 21 days sac , the only difference being the thicker fibrin laminae lining the sinuses , and the more abundant

fibrinous deposit among the decidual cells (fig. 21).

Foetal Placenta :- This presents very striking changes from normal , these changes being of the same nature as , but of a more advanced character , than those noted in Experiment II. There we noted that the degeneration of the foetal ectoderm was most advanced in the centre , and in the sac at present under consideration we find that in the central area differentiation of tissue is almost completely lost. In several areas the ectoderm is represented only by an irregular , darkly stained purple mass (fig. 26). In places traces of the original columns , into which the placenta was divided , are visible , but nowhere are cell outlines visible. In this area also there are large areas of blood extravasation. Towards the foetal surface the degeneration is not so marked , the nuclei in many cases taking on the stain well , but the protoplasm shows granulation. Towards the maternal surface there is a considerable deposit of fibrin , and the ectodermal cells have almost completely disappeared (fig. 21).

The mesoderm at the foetal surface of the placenta is greatly increased in thickness and density. In several areas there is an extensive deposit of fibrin (fig. 26).

Foetus :- The epithelial covering is everywhere shed , no trace being left. The connective tissue is of an entirely different character from normal. The cells are rounded in

shape , owing to the breaking down of the protoplasm in their peripheral parts and branching processes , and to the swelling of the nuclei which have also undergone extensive granular degeneration (fig. 30). The ground substance is composed of coagulated lymph and the debris from the broken down protoplasm of the connective tissue cells and blood corpuscels. The tissue as a whole shows a more advanced stage of degeneration than that observed in the foetus of the previous experiments , the granular degeneration of the nuclei being specially pronounced.

The heart has undergone very considerable degeneration of the same nature as that noted in the previous experiments. The cell outlines have been lost , the protoplasm is swollen and stains irregularly , while many of the nuclei have disappeared , and those that remain stain faintly and show granular degeneration.

The liver is very much broken up , so that the arrangement of the cells in columns is scarcely recognisable. The protoplasm of the cells is very much swollen and has begun to break down at the periphery. The nuclei are extremely granular and many have entirely disappeared. Between the degenerated liver cells is a mass of debris derived from the breaking down protoplasm and from the degenerated and shrivelled blood corpuscles. In this the round connective tissue

cells are visible with swollen granular nuclei.

The kidney shows less extensive and advanced degenerative changes than the liver. The glomeruli and the tubules are still almost perfactly formed and are quite recognisable as such. There is however no blood seen in the glomeruli and they are compressed , so that the tuft comes close up to the inner surface of the capsule , the epithelium of which is breaking down. Throughout there is an increase in the connective tissue cells with degenerated nuclei. The epithelium of the tubules stains irregularly , and the protoplasm at the free margin of many of the cells is breaking down and filling the lumina. The nuclei are deeply placed and show more marked granulation than in the two previous experiments. There is an apparent increase in the number of connective tissue cells in the stroma , and they , here as elsewhere , are undergoing granular degeneration.

Membranes :- The yolk sac wall has in most places lost its epithelial covering , and where present , it is degenerated. The cells have run together and the nuclei are swollen , so that buds of multinucleated protoplasm are produced , the nuclei showing granular degeneration. The mesodermic tissue is infiltrated with lymph , and there is much debris from the breaking down of the connective tissue cells; those that remain show the same degeneration as that described as

occurring in the corresponding cells of the foetus. The red blood corpuscles are shrivelled and have lost their nuclear staining , and many are quite broken down.

Second Punctured Sac from Right Horn - removed post mortem.

The wall at the placental site is 1.3 mm. in thickness. Opposite the placenta it is somewhat thinner , owing to the distension of the sac by the foetus of the adjoining sac. The mucosa is flatter than in the first sac , possibly for the same reason. Giant cells are numerous. In one or two places , especially in the lateral areas , there is evidence of glandular and epithelial proliferation. There are no cushions of decidual tissue , the stroma of the mucosa presenting a dense appearance.

Maternal Placenta :- This presents exactly the same characters as the preceding , consisting for the most part of fibrin tissue with degenerated and compressed decidual cells , leucocytes , and phagocytes embedded in it , with a few large maternal sinuses surrounded by uninucleated and multinucleated decidual cells , and lined by laminae of fibrin.

Foetal Placenta :- This also presents exactly the same features as were noted in the preceding sac , the one description applying accurately to the other. In the present sac there is , if anyhting , more blood extravasation.

Foetus :- The degenerated foetus is lying on the surface

of the foetal placenta. The organs and tissues show the same degenerative change as that detailed in the left horn sac.

SUMMARY OF RESULTS OF EXPERIMENT III.

There has been no re-secretion of the fluid. The muscular coats show the same hypertrophy as in the previous experiments. The foetus has died and has begun to degenerate , while the changes in the placenta , both in the maternal and foetal portions are of the same nature as those previously noted - the maternal placenta tending to develop along the normal lines with a greater amount of fibrin deposit , and the foetal stedily degenerating in its cellular elements , and showing an increased deposit of fibrin in the mesoderm. Its area has increased since the time of operation.

EXPERIMENT IV.

Operation when 21 days pregnant.

Animal killed when 28 days pregnant.

Interval = 7 days.

Operation :- On opening the abdomen there were found to be five sacs in each horn. One of the sacs in the right horn was punctured , and .8 cc. of clear fluid withdrawn. In the left horn the sac next the vagina was completely excised for control , and the sac next it was aspirated and also the second terminal one , 1 cc. of clear fluid being got from each.

The animal made a perfect recovery and was killed 7 days later , it then being 28 days pregnant.

At the post mortem examination the abdomen was found to be perfectly healthy; there was no effusion and no lymph deposit. In the right horn of the uterus there were found four large sacs containing an abundance of fluid , and corresponding in size to those usually seen at the 28th day. The sac which had been aspirated was very much smaller - little if any larger than at the time of operation , and it contained only a few drops of fluid.

In the left horn were two large well formed sacs with a

large quantity of fluid , while the two which had been aspirated had remained of the same size as at the time of operation , and contained only a very small quantity of fluid (fig. 9).

DETAILED DESCRIPTION OF SACS.

1. Naked eye Appearances.

Sac Excised at Operation.

This is ovoid in transverse section measuring 2.5 cm. in the vertical by 2.cm. in the transverse diameter. The placenta is 1 cm. in depth , the maternal part being .4 cm. , and the foetal .6 cm. , while the space between its surface and the roof of the sac is 1.4 cm. In the maternal placenta the zone of separation is clearly marked as a translucent band next the uterine wall; the rest of this portion is pale in colour , with a few darker , more vascular areas. The foetal placenta is red and vascular (fig. 10,A).

The foetus is 4 cm. in length , is of a translucent pink colour , and is perfectly formed with limbs and digits complete (fig. 10,B).

Normal Sac from Right Horn - removed post mortem.

It is ovoid in outline measuring 3.5 cm. in vertical , by 2.5 cm. in transverse diameter. The

placenta is 1 cm. in depth , .3 cm. of this being maternal , and .7 cm. , foetal. The space above the placenta in which the foetus lies is 2.3 cm. and contains 2 cc. of amniotic fluid. In the maternal placenta the zone of separation is more clearly differentiated than in the sac removed at operation , and the maternal placenta is thinner and paler in colour. The foetal part is red and vascular (fig. 10,C).

The foetus is 7 cm. in length , of a pink colour , and having almost the appearance of a full time rabbit (fig. 10,D).

Distal Punctured Sac from Left Horn - removed post mortem.

On opening the cornu the placenta and membranes with the foetus inside were found lying free in the sac , the rough surface on the mesometric aspect of the uterine wall from which it had been detached being quite visible. The foetus was lying with its ventral surface towards the placenta , and was compressed laterally between the head of the foetus on its distal side , and the hind quarters of that on its proximal aspect. There was no fluid in the amniotic sac and the whole ovum , placenta and foetus , was of a dull grey colour (fig. 10,F). The foetus is 3.5 cm. in length, and has therefore attained only the development of that contained in the sac removed at the time of operation. The placenta in transverse section is .7 cm. in depth , .3 cm. being materbal , and .4 foetal. Both the portions of the

placenta are dull grey in colour , but the distinction between the maternal and foetal is still evident , owing to the slightly greater vascularity of the latter.

Proximal Punctured Sac from Left Horn - removed post mortem.

In this sac the roof was cut away after hardening in formalin , and the relation of the foetus in its membranes is shown in fig. 10,E. It will be seen that the amniotic sac closely invests the foetus and contains only a few drops of fluid. The foetus only measures 3.5 cm. in length , and therefore has not grown any since the time of operation. It is of a dull grey colour , is soft , and has obviously been dead for some time. The placenta is .8 cm. in depth , the maternal part being .4 cm. , and the foetal .4 cm. In the maternal placenta the zone of separation is very well marked - even more so than in the normal 28 days placenta. The rest of the placenta is pale in colour. The foetal part is red and vascular , but not so much so as the normal , and it is of denser consistence.

Punctured Sac from Right Horn - removed post mortem.

The vertical diameter is 2.5 cm. and the transverse diameter 2 cm. The placenta is 1 cm. in thickness , .6 cm. being foetal and .4 cm. maternal. The space between the placenta and the roof of the sac is 1.2 cm. , and this is completely filled up by the foetus , only

a few drops of fluid being present in the amnion. The zone of separation in the maternal placenta is very distinct as in the other punctured sacs , the rest of it being pale in colour. The foetal placenta is paler than normal , but the distinction between it and the maternal is very evident.

The foetus is dull grey in colour , is soft , and has lost its translucent appearance. It has not been flattened out. It is 4 cm. in length - the same as the foetus removed at the time of operation , to which it corresponds in development.

2. Microscopic Examination.

Normal Sac from Left Horn - excised at operation.

The microscopic appearances of this sac correspond in every respect to those detailed in the normal 21 days sac of the preceding experiment , and need not here be described.

Normal Sac from Left Horn - removed post mortem.

The wall of the sac is .5 mm. in thickness. There is a considerable quantity of loose cellular tissue between the two muscular layers. The mucosa is flattened and covered with a single layer of columnar epithelium which is little folded. There are no giant cells present.

Maternal Placenta :- The zone of separation consists of a very fine reticulum composed of shrunken uninucleated decidual cells with a few fibrin threads scattered among them. The vessels in it are small and have slightly swollen endothelium lining them. The rest of the placenta is made up for the most part of fibrin and coagulated lymph , in the midst of which lie , island-like , the large uterine sinuses with their perivascular sheaths of multinucleated decidual cells. The latter are shrunken and compressed by the fibrin deposit , and a little way beyond the vessel they have almost disappeared. The fibrin tissue is nowhere very dense. In it lie the remains of the decidual cells and numerous multinucleated giant cells.

Foetal Placenta :- Consists of large foetal capillaries containing blood , arranged in columns and consisting practically of an endothelial lining. They are covered by a thin layer of plasmodial ectoderm , which is however in places deficient, so that the foetal vessels are directly bathed in the maternal blood. The ectoderm , although thin and attenuated , shows no sign of degeneration. It is entirely plasmodial , but the nuclei , where present , stain well and show no granulation. The mesoderm is a fine reticulum , nowhere very abundant. The larger vessels in it have thick walls. There is no fibrinous deposit in it.

Foetus :- It is fully formed - all the organs and tissues have the normal appearance of the new born animal.

Distal Punctured Sac from Left Horn - removed post mortem.

The placenta and foetus in this sac have become detached from the uterine wall. Sections of the latter were cut separately through the rough surface from which the separation had taken place. The wall is thickened - measuring 1.2 mm. , as compared with the normal .5 mm. This thickening is due to the contraction of the muscular layers , the fibres of which have a folded , wavy appearance. The mucosa is entirely wanting over the area to which the placenta had been attached , and in its place is a ragged surface to which some of the shrunken decidual cells from the zone of separation are still adherent. There is no attempt at the covering of this area by epithelium.

Maternal Placenta :- The zone of separation is more finely reticulated than the normal in consequence of the cells being more shrunken. The fibrinous deposit in it is very slight (fig. 17). It has given way close to the uterine wall , so that the greater part remains attached to the maternal placenta , only a few cells , as mentioned above , being left adherent to the uterine wall. The rest of the placenta in general features corresponds to the normal 28 days placenta just described , but differs from it in the greater abundance

of the fibrin deposit and its denser character (fig. 22), so that the decidual cells are more compressed and atrophied; many of the sinuses are filled with coagulated blood. The cellular elements present in the fibrinous deposit are of the same character as those of the normal placenta , except that the recognisable remains of decidual cells are fewer.

Foetal Placenta :- The departures from normal are more striking in this than in the maternal portion. The mesoderm is more abundant than normal , and is invaded by fibrin threads , while the cells are beginning to undergo granular degeneration of their nuclei. This fibrin invasion is not so extensive as that present in the punctured sac of the preceding experiment in which the lapse of time between the operation and the death of the animal was the same , but in which the operation was done at an earlier stage of pregnancy. The fibrinous deposit can be traced wherever the mesoderm penetrates into the ectoderm. The arrangement of the ectoderm into columns has attained to the degree usually seen in the 25 days placenta , the columns not being so fine as that of the 28 days one. For the most part the ectoderm stains well and shows no sign of degeneration , and the nuclei are perfectly formed. There is no tendency to extension into the maternal placenta (fig. 22). In one or two places near the maternal and foetal junction large areas of ectoderm

have been invaded by fibrin , and almost completely replaced by the latter. Near such areas the remaining ectoderm is degenerated , the protoplasm staining irregularly , and the nuclei having entirely disappeared. The foetal blood is much degenerated; the corpuscles are irregular in shape and shrivelled , while they stain very faintly - ghost corpuscles. In the maternal vessels the blood is quite normal in appearance. The general effect of the degeneration of the foetal corpuscles is to give the whole foetal placenta a very abnormal appearance , but examination of the individual elements shows that the departures from normal are comparatively slight.

Foetus :- The epithelium is entirely absent over the greater extent of the surface , and where present , is raised up , and the cells show degenerative changes in the nuclei which are swollen , indistinct and granular.

The connective tissue has lost its normal myxomatous appearance , and is made up of a ground substance of coagulated lymph and debris , while the cells are rounded with swollen granular nuclei. The granulation is not so marked as in the foetus from the punctured sac in the preceding experiment. The endothelium has stripped off the blood vessels. The cartilage has the general character of that of a 21 days foetus; only at one or two places in the vertebrae has bone

formation begun. The matrix shows no pathological change , but the cells are shrivelled , and the nuclei swollen and granular (fig. 42).

Muscle cells where present show granulation of the protoplasm , and very faint staining of the nuclei (fig. 42).

The liver is very extensively degenerated (fig. 38). The tissue is broken up so that the arrangement of cells in columns is lost. Many of the cells lie isolated from each other , and between those which are still in contact no cell outlines can be distinguished. The protoplasm is granular and broken down. In some the nucleus has disappeared , and in the others it is swollen and granular and stains very faintly. Between the liver cells is an aggregation of debris formed by their broken down protoplasm and the remains of blood corpuscles. In it there are a few giant cells. No blood vessels are distinguishable and no normal blood.

The stomach wall is made up of the same sort of connective tissue with degenerated cells as is present elsewhere. The epithelium has been cast off and is lying in the cavity. The nuclei of the cells are much swollen and very granular.

The kidney contains many round cells with granular nuclei. The epithelium of the tubules is swollen , the cell protoplasm stains badly and is beginning to break down at its free margins so that the luminae are filled with debris. In some

of the tubules the entire lining has been thrown off. The nuclei stain faintly and are extensively granular. The glomeruli are shrunken and contain round cells , and are in consequence dense , solid looking structures in which no blood is visible and which come close up to the capsule. The space between the kidney elements proper is occupied by round cells , broken down blood corpuscles and cellular debris.

There is no effusion into the peritoneal cavity.

The lungs are dense solid structures. The connective tissue is of the same character as elsewhere , and the epithelial lining of the bronchi is swollen and undergoing granular degeneration. There is no blood effusion into the lungs or pleura.

Membranes :- The yolk sac is invaded by fibrin. There is no normal blood present in it , and the epithelium is for the most part shed , and where present , is undergoing granular degeneration.

Punctured Sac from Right Horn - removed post mortem.

The uterine wall is slightly thicker than normal , owing to retraction of the muscular layers. There is no proliferation of the epithelium or sub-mucous tissue of the lining mucosa.

Maternal Placenta :- The zone of separation is very well marked and its structure is more attenuated than in the normal

28 days sac. There is no fibrinous deposit in it. In several places is has become detached from the uterine wall , and the whole placenta is evidently on the point of being cast off. The rest of the placenta exactly resembles that of the distal punctured sac of the left horn just described; there is the same excess of fibrin tissue with atrophy of the cellular elements and thrombosis of vessels as was noted in it.

Foetal Placenta :- The mesoderm is extensively invaded by fibrin as is also the ectoderm in the deeper parts where it also shows signs of degeneration. The general structure resembles that usually seen at the 25th day.

Foetus :- The degeneration is of exactly the same nature and extent as that of the foetus of the punctured left horn sac just described.

SUMMARY OF EXPERIMENT IV.

In all the punctured sacs the foetuses have died shortly after the operation as they show the same stage of development as the one removed at the time of operation.

In no case has there been any re-secretion of the liquor amnii.

The changes in the placentae of the punctured sacs have been comparatively slight. The maternal placenta has gone on developing in the normal way , except that the fibrinous deposit has been more extensive , so that at the 28th day - the time at which the animal was killed - it resembles a 30th day placenta , and in the case of one of the sacs it has become detached through the much attenuated zone of separation and lies free in the cavity.

In the foetal placenta there has been fibrinous deposit in the mesoderm throughout , and in the deeper parts of the ectoderm where the trophoblast has degenerated. The degen-generative changes in the foetal placenta are not so marked as in the previous experiment where the interval between the operation and the death of the animal was the same , but in which the fluid was aspirated at an earlier stage of gestation.

The degenerative changes in the foetus are of the same nature as those previously described , viz. , a granular degen-

eration of all the cellular elements. The stage to which this degeneration has proceeded is not so advanced in this as in the previous experiment. This is especially noticeable in the liver , but is also apparent in all the other tissues. This is to be accounted for by the more advanced development of the foetus at the time of its death.

EXPERIMENT V.

Operation when 16 days pregnant.

Animal killed when 25 days pregnant.

Interval = 9 days.

Operation :- On opening the abdomen there were found to be three gestation sacs in each horn. The distal sac of the left horn was punctured , and 1.5 cm. of clear fluid withdrawn , the sac being left quite flaccid. In this case no sac was excised , this being one of the first experiments performed , and at the time it was thought that it might produce abortion.

The animal recovered completely from the operation and was killed 9 days later , it being then 25 days pregnant. On post mortem examination the right horn was found to contain three apparently normal sacs , each distended with fluid. The two proximal sacs of the left horn presented a similar appearance , only differing in being slightly smaller in size. The distal sac of the left horn was very much smaller than any of the others , and on external palpation was hard , and apparently contained only a few drops of fluid.

DETAILED DESCRIPTION OF THE SACS.

1. Naked eye Appearances.

Normal 16 days Gestation Sac.

As stated above a sac was not removed at the time of operation , but the following are the normal appearances and measurements of the 16days sac. It measures about 2.9 cm. in vertical , and 2.4 cm. in transverse diameter. The placenta is 1 cm. in thickness , foetal and maternal portions being of about equal size. The space between the placenta and the roof of the sac is about 1.7 cm. The foetus is from 1.2 to 1.5 cm. in length. For a detailed description of the placenta and foetus see Experiment I.

Normal Sac from Left Horn - removed post mortem.

It is ovoid in shape measuring 3.2 cm. in vertical , and 3 cm. in transverse diameter. The placenta is .3 mm. in depth , 3 mm. of this being maternal and 5 mm. foetal. The space between the placenta and the roof of the sac is 2.2 cm. The foetal is readily distinguished from the maternal placenta by its greater vascularity , and as noted , it is of nearly twice the thickness of the latter. There are no haemorrhages visible. The zone of separation is distinct. The foetus is perfectly developed , and measures 6.5 cm. in length. The normal sac from the left horn resembles in every respect the foregoing , but is of a slightly

larger size. The foetuses are of equal size.

Punctured sac from Left Horn - removed post mortem.

The sac is almost circular in outline , measuring 2 cm. in the vertical , and 1.9 cm. in the transverse diameter. The placenta is 1.2 cm. in depth , and the space between it and the roof of the sac only 4 mm. This space is occupied by a reddish brown gelatinous material , in which the remains of the foetus are embedded (fig. 11). The uterine wall is very considerably thickened , measuring 1.5 mm. at the placental site. The placenta is of a dense fibrosed appearance , pale in colour in comparison with the normal , the foetal part being a little less so than the maternal. In the latter some haemorrhages are visible. The foetal and maternal portions are equal in depth. The zone of separation is very well marked - more so than normal. The foetus is represented by a greenish flattened body lying embedded in the gelatinous material.

2. Microscopic Examination.

Normal 16 days sac

This presents the characters of the normal sac removed at operation from the animal of Experiment I , and no further description need be given here.

Normal Sac from Left Horn - removed post mortem.

In the uterine wall the distinction between the two muscular coats is well marked , large vessels being situated between them. The mucosa is flattened; the connective tissue is abundant with mucous glands in it.

Maternal Placenta :- This is small in extent only being about half the depth of the foetal. In the zone of separation the tissue is very loose , the cells being shrunken and many having disappeared altogether. The rest of the maternal part is made up of fibrin tissue which is denser than at the 16th day. Embedded in it are the remains of decidual cells , polynuclear giant cells and leucocytes. Round some of the sinuses are sheaths of multinucleated decidual cells , many of which , however , show signs of degeneration - absence of nuclei and shrinking of the cell body. The sinuses are surrounded by many layers of fibrin , and within this , in some cases , the swollen endothelium persists. There are no haemorrhages present.

Foetal Placenta :- This is very vascular consisting of columns of foetal and maternal vessels , separated from each other by very thin layers of foetal ectoderm , so that the two blood systems are in close apposition. The ectoderm everywhere stains well and shows no tendency towards degeneration , and at the junction with the maternal tissue it still retains

its power of extending along and enclosing maternal blood spaces. The mesoderm while fairly abundant at the foetal surface , is not visible in the deeper parts , all the space between the ectodermic columns being occupied by the blood vessels. Where present it presents a denser appearance than at an earlier stage of pregnancy , but there is no fibrinous deposit in it.

Punctured Sac from Left Horn - removed post mortem.

The wall is thick , measuring 1.5 mm. at the placental site. The muscular layers are compact , the distinction between the two coats not being well marked. Opposite the placental attachment a number of large uninucleated giant cells , somewhat smaller than those usually present in the obplacental folds , are situated among the fibres of the inner muscular coat , and between it and the outer coat. They have the same general appearance as the ordinary giant cells , except that the nucleus is larger in proportion to the size of the cell body. The mucous membrane is very considerably thickened and hypertrophied , and it is raised into villous processes at the points where these are normally present in the non-pregnant uterus. The submucous tissue is dense and cellular; glands are abundant , and the surface epithelium is undergoing rapid proliferation.

Maternal Placenta :- The zone of separation is an extremely

loose tissue composed of shrunken decidual cells. There are a few fibrin threads between them. Blood vessels are few and small. The rest of the placenta has the general character of the normal 25 days sac already described , but presents the following slight differences. It is greater in depth , measuring 5 mm. as compared with 3 mm. It is however smaller in area as it is compressed laterally. The fibrin tissue is denser than normal , and the islands of decidual cells show more advanced stages of degeneration and shrinkage , the nuclei having entirely disappeared from the majority of them.

It has advanced in development from the 16th day as shown by the dilated sinuses which approach up to the face of the foetal placenta , and which are lined by thick laminae of fibrin and have lost their endothelium. The fibrin has been deposited round these sinuses just as it is in the normal development of the placenta.

Foetal Placenta :- This shows changes from the normal of the same character as , but more advanced than , those described in the previous experiments. The most striking change is the large quantity of fibrin which is deposited , not only in the mesoderm at the foetal surface , as has been noted in the punctured sacs in the previous experiments , but also throughout the whole area of the foetal placenta. The

deposit is densest at the foetal surface and towards the maternal junction , and in the former region many of the vessels are fibrosed. This fibrin tissue has in many places entirely replaced the foetal ectoderm over large areas. In such places faintly staining masses of protoplasm , the remains of the ectoderm , are seen , and here and there a group of decidual cells , many of which have lost their nuclei , and all of which are shrunken. From these larger denser areas of fibrinous deposit smaller and less dense strands reach out , and invade the rest of the placenta , breaking up the ectoderm into islands. In some parts , especially towards the lateral borders of the placenta the fibrin strands are very fine , and the original structure of the placenta is still retained , but even in such areas the nuclear staining of the ectoderm is almost entirely lost , and it appears as a homogeneous protoplasm , interposed between the columns of maternal and foetal vessels. Towards the centre of the placenta , even in areas not invaded extensively by fibrin , the ordinary appearances are entirely lost , no division of the ectoderm into columns being visible , the whole appearing as a granular , purple-stained , solid mass. Throughout the placenta there are a few recent haemorrhages as well as the fibrin deposit.

The gelatinous material filling up the sac between the placenta and the roof is a homogeneously staining substance

without structure. It is probably mucus derived from the glands , and in it the degenerated foetus is embedded.

Foetus :- The minute details of the structure of the various organs and cellular elements are obscured by the depth of staining of the mucous material in which the foetus lies embedded. The general character of the degenerative change is the same as that described in the other experiments. The cellular tissue has lost its myxomatous character and the cells have become rounded and granular. The heart fibres are broken up and nuclear staining is deficient.

The liver has lost its columnar structure in consequence of the cells breaking down . There is a great deal of debris between them , and everywhere cell outlines are lost and nuclear staining , granular or absent.

The kidney has very much the appearance of that described in the degenerated foetus of Experiment III. The changes in it are not so advanced as in the liver , as for the most part the epithelial lining of the tubules is still intact although the cells are swollen and nuclei granular.

Membranes :- There is a deposit of fibrin in the mesoderm of the vascular wall of the yolk sac and the epithelial covering has been lost. The amnion closely invests the foetus and contains no fluid.

SUMMARY OF EXPERIMENT V.

The liquor amnii was aspirated and there has been no re-secretion.

The foetus shows the same stage of development as that of the one removed at operation. It must therefore have died very soon after the aspiration of the fluid. The degenerative changes which it has undergone are of the same nature but more advanced than, those noted in the previous experiments. The maternal placenta has gone on growing and developing so that it has advanced from the normal condition present at the 16th day to that usually seen at the 28th. The only points in which it differs from normal are the greater amount of fibrinous deposit , the greater attenuation of the tissue forming the zone of separation , and the greater depth owing to the foetal placenta having ceased to invade it.

In the foetal placenta there has been a fibrinous deposit in the mesoderm , and to a less extent this has invaded the ectoderm. The latter has lost its power of penetrating the maternal tissues , and has undergone very marked degenerative changes , so that nuclear staining is deficient and in some places entirely absent , and the protoplasm is granular and vacuolated.

EXPERIMENT VI.

Operation when 10 days pregnant.

Animal killed when 20 days pregnant.

Interval = 10 days.

Operation :- On opening the abdomen there were found to be two sacs in the right horn and four in the left. Both of the right horn sacs were punctured , and .75 cc. of fluid with some flocculent deposit in it withdrawn from each. In this case no sac was excised.

The animal recovered perfectly from the operation , and was killed 10 days later , it then being 20 days pregnant. The abdomen was perfectly healthy - no signs of sepsis and no fluid , but a few adhesions being present. In the left horn of the uterus were four well developed sacs containing abundance of fluid , while in the right horn were the two sacs which had been punctured , little if any larger than they were at the time of operation. To the touch they were firm and resisting , and contained only a very small quantity of fluid.

DETAILED DESCRIPTION OF THE SACS.

1. Naked eye Appearances.

Normal 10 days Sac.

This is oval in outline measuring 1.1 cm. in vertical by 1 cm. in transverse diameter (fig. 1). The placenta is .6 cm. in depth , 4.5 mm. of this being maternal , and 1.5 mm. foetal. The space between the surface of the placenta and the roof of the sac is 4 mm. The maternal placenta is a dense , firm looking , whitish tissue with numerous red vascular points throughout. No zone of separation is distinguishable. The division into two lobes is quite evident. The foetal placenta is distinguishable as a thin , more vascular area , covering the convex surface of each lobe. The foetus is hardly distinguishable in transverse section naked eye.

Normal Sac from Left Horn - removed post mortem.

The sac is ovoid in shape measuring 2.8 cm. in vertical by 2.5 cm. in transverse diameter. The placenta is 1 cm. in depth , of which .4 cm. is maternal and .6 cm., foetal. The space between the surface of the placenta and the roof of the sac is 1.8 cm. The foetal and maternal portions are easily distinguishable by reason of the greater vascularity of the former. In the maternal portion a faint zone of separation next the uterine wall can

be distinguished. The foetus is 3.1 cm. in length; the abdomen is closed and the limbs and digits perfectly formed.

Distal Punctured Sac from Right Horn - removed post mortem.

The sac is almost circular in outline, the greatest vertical diameter being 1.4 cm. and the greatest transverse 1.4 cm. The placenta is .8 cm. in depth , and the space between it and the roof of the sac .3 cm. The uterine wall is thicker than that of either the 10 days or the 20 days sac. The distinct division of the placenta into two lobes is no longer visible , owing to the compression from side to side , so that only a small notch is seen at the free surface. The whole placenta is dense looking of a greyish yellow colour , with redder , more vascular areas scattered about. No differentiation into maternal and foetal portions is evident. The zone of separation which appears as a clearer convoluted band next the uterine wall is much more distinct than in the normal 20 days sac. The space between the surface of the placenta and the roof of the sac is occupied by a grey , gelatinous substance , and no foetus can be distinguished. There was no fluid.

Proximal Punctured Sac from Right Horn - removed post mortem.

This sac is slightly larger than the preceding and more ovoid in outline , measuring 1.6 cm. in vertical by 1.5 cm. in transverse diameter. The placenta

is .9 cm. in depth , and the space between it and the roof of the sac .3 cm. The general appearance of the placenta is like that of the preceding , no distinction between maternal and foetal portions being visible , and the zone of separation being very well marked. The division of the placenta into lobes is slightly more evident as the notch extends a little deeper. There was no fluid present.

2. Microscopic Examination.

Normal 10 Days Sac.

The uterine wall is .5 mm. in thickness. The two muscular coats are separated by loose cellular tissue containing blood vessels. The outer longitudinal coat is twice the thickness of the inner circular layer. The mucous membrane is flattened out , so that original folds are no longer distinguishable. The submucous tissue is cellular and very vascular , and the epithelial lining is a single layer of low columnar cells. There are no giant cells present.

Maternal Placenta :- The region of the uterine sinuses is made up of uninucleated decidual cells arranged as perivascular sheaths round the dilated uterine sinuses. The latter are filled with maternal blood and are lined by endothelium which is slightly swollen. Fasciculi of the origin-

al corium run between the islands of decidual cells. There is no zone of separation differentiated , and there is no fibrin deposit anywhere in this part of the placenta. In the intermediary region the sinuses are more dilated , their endothelial lining more swollen , and the perivascular sheaths are composed of multinucleated decidual cells. There is some fibrin deposit round them , and multinucleated masses of protoplasm - the remains of the uterine glands - lie as islands on the tissue.

Foetal Placenta :- This is still only of small extent. The ectoderm has extended some way into the intermediary region along the obliterated glands , and has surrounded some of the sinuses of the intermediary region which have lost their endothelial lining. It is partly plasmodial. At the surface there is a good deal of mesodermic tissue with foetal blood vessels containing nucleated blood. This mesoderm is extending down into the ectoderm , and breaking it up into columns.

Foetal Membranes :- The foetus is seen to be completely surrounded by the amnion which is yet only separated from the former by a narrow space all round (fig. 1). The vascular wall of the yolk sac is readily distinguished , the vessels in it containing nucleated blood. The non-vascular wall is seen running round the uterine cavity closely apposed to

the mucosa , and fixed to it here and there by cellular buds from its outer surface. The allantois conveying the foetal vessels to the placenta is also seen (fig. 1). The greater part of the space above the placenta will thus be seen by reference to fig. 1 to be occupied by the cavity of the yolk sac , the part nearer the placenta being occupied by the extra embryonic body cavity. As the puncture was made in the upper part of the sac at the operation the cavity which was aspirated was the yolk sac.

Normal 20 dys Sac from Left Horn - removed post mortem.

No detailed description of this need be given here as it corresponds almost exactly to the 19 days sac described in Experiment I. It need only be noted that in the maternal placenta a zone of separation is beginning to differentiate; that the intermediary region , as such , has disappeared; that the uterine sinuses are larger and many of them have lost their endothelial lining , and are now lined by fibrin laminae , while there is an extensive fibrinous deposit round and among the perivascular decidual cells. The foetal placenta is made up of columns of ectoderm , most of which is plasmodial. The non-vascular wall of the yolk sac has disappeared , and the amniotic cavity fills up the free space of the sac.

Distal Punctured Sac from Right Horn - removed post mortem.

The uterine wall is 1 mm. in thickness. The two muscular layers are closely apposed , there being very little cellular tissue between them , and no large vessels. The mucosa is for the most part flattened , but in the lateral areas it is raised into villous processes by the proliferation of the sub-mucous tissue and surface and glandular epithelium. There are no giant cells present.

Maternal Placenta :- This is made up for the most part of fibrin tissue and debris. There is a distinct zone of separation next the uterine wall made up of an extremely fine cellular reticulum , many of the cells of which are shrunken and shrivelled. The vessels in it are small and have slightly swollen endothelial linings. There are no fibrin threads between the cells. Beyond the zone of separation the tissue is one mass of fibrin with only a few cellular elements embedded in it. The strands of the original corium cannot be distinguished. The sinuses are much larger than those of the 10 days placenta , but most of them are fibrosed. Those that are patent are lined by fibrin laminae , while in some the swollen endothelium which has been cast off is still seen in the lumen of the vessel. Round some of these vessels , especially towards the foetal surface , the remains of multinucleated decidual cells are visible. Towards the free surface a band of tissue somewhat similar to that making

up the zone of separation is present. In it there is no fibrin , and it is lined on its free surface by a single layer of columnar epithelium , similar to that lining the uterine cavity. Here and there this epithelium extends down into the placenta in the form of tubular glands.

There is not a trace of foetal structures anywhere to be seen - no ectoderm and no traces of the foetal membranes of foetus - although the whole extent of the placenta was cut serially.

Proximal Punctured Sac from Right Horn - removed post mortem.

The microscopical appearances of the sac and placenta are identical with the foregoing. No trace of foetus or foetal placenta is visible.

SUMMARY OF THE RESULTS OF EXPERIMENT VI.

In this experiment the operation was performed at an earlier stage of gestation than any of the preceding. At the 10th day the amnion , although completely formed , has only a small cavity , and contains little fluid , so that the fluid which was withdrawn was the yolk sac fluid. This has resulted in the complete disappearance of the foetus , and of the foetal portion of the placenta which had just begun to form. No comparison can therefore be drawn between the changes in it and those induced in the other experiments. Unfortunately I have been unable to operate on another animal at the same stage. The changes in the maternal placenta are of the same nature as those hitherto noted. The placenta has gone on developing along the normal lines , so that in general appearance it resembles the normal 20 days placenta, differing from it however , in the larger quantity of fibrin tissue present. The zone of separation has become differentiated. The uterine sinuses have enlarged , and have lost their endothelial lining , which has been replaced by fibrin laminae , while the decidual cells have replaced the original corium.

EXPERIMENT VII.

Operation when 15 days pregnant.

Animal killed when 27 days pregnant.

Interval = 12 days.

Operation :- On opening the abdomen there were found to be three gestation sacs in the right horn and four in the left. Two of the latter were punctured - the one next the tube and the one next the vagina - and 1.5 cc. of fluid withdrawn from each , while a little more escaped on withdrawing the needle. The abdomen was then closed.

After an interval of 12 days the animal was killed , it being then 27 days pregnant , and the uterus excised. In the right horn were three large well developed sacs , each tense with fluid. The two middle sacs of the left horn presented the same appearance , and were of the same size. The two terminal sacs - tubal and vaginal - of the left horn , which had been aspirated were very much smaller in size , and seemed to be entirely filled with the placenta , as they felt firm and had not the tense elastic consistence of the others.

DETAILED DESCRIPTION OF THE SACS.

1. Naked eye Appearances.

Normal 15 days Sac .-

No sac was excised in this case at the time of operation , but the following are the normal measurements and appearances of a sac at the period of pregnancy at which this experiment was carried out. It measures 2.8 cm. in vertical by 2.4 cm. in transverse diameter. The placenta is 1 cm. in thickness , maternal and foetal parts being equal in size , while the space between them at the roof of the sac is 1.5 cm. The foetus is 2.1 to 2.3 cm. in length, and its limbs are formed.

Normal Sac from Left Horn - removed post mortem.

The sac is ovoid in shape measuring 4 cm. in vertical , and 3.5 cm. in transverse diameter. The placenta is 1 cm. in thickness , of which .4 cm. is maternal , and .6 cm. foetal. The space between the top of the placenta and the roof of the sac is 2.9 cm. The maternal placenta is pale in colour in contrast to the foetal , so that the two are readily distinguished. There are no haemorrhages visible. The zone of separation is very well marked. The foetus is fully formed , being within three days of full time , and measures 7.5 cm. in length. The normal sacs from the right horn presented the same appearances and were

of the same size.

Punctured vaginal Sac from Left Horn - removed post mortem.

The sac is ovoid in form with the long diameter transverse , and measuring 2.5 cm. , while the vertical is 2 cm. (fig. 12). The wall is markedly thickened. The placenta is 1.4 cm. in depth , and the free space between it and the roof of the sac is entirely filled by the flattened remains of the foetus which is only 1 mm. in thickness. The general colour of the placenta is grey with a slightly redder area in the centre which may be hameorrhagic. The distinction between maternal and foetal portions is entirely wanting. The zone of separation is very clearly marked. The foetus is represented by the flattened grey mass lying on the surface of the placenta.

Punctured tubal Sac from Left Horn - removed post mortem.

This sac is almost circular in outline , measuring 2 cm. in vertical by 2.1 cm. in transverse diameter. The placenta is 1.2 cm. in depth , and the space between it and the roof of the sac is 5 mm. The general appearance of the placenta is exactly the same as that described immediately above , except that the haemorrhagic area in the centre is somewhat more extensive. The foetus is represented by a flat , greyish mass lying on the surface of the placenta , and measuring .9 cm. in length by .4 cm. in

thickness.

2. Microscopic Examination.

Normal 15 days Sac.

This is in all essentials similar to the 16 days sac described in Experiment II , and need not be further described.

Normal Sac from Left Horn - removed post mortem.

The wall presents the same characters as described in the normal sacs in the preceding experiments , except that the giant cells have entirely disappeared. The thickness is .5 mm.

Maternal Placenta :- The zone of separation is a very finely reticulated tissue with shrunken and degenerated cells , many of which have lost their nuclei. The sinuses are small and lined with thickened endothelium. The rest of the maternal placenta consists of large uterine sinuses which approach right up to the face of the foetal placenta , surrrounded by multinucleated giant cells which are surrounded by fibrin threads. Beyond these islands of decidual cells are extensive deposits of coagulated lymph and fibrin with the degenerated remains of decidual cells embedded in them. The whole maternal placenta thus practically consists of fibrin tissue containing fibrin- lined sinuses surrounded by degenerated decidual cells.

Foetal Placenta :- This consists of columns of wide foetal capillaries consisting practically of an endothelial wall. These are covered by a thin layer of plasmodial ectoderm , which separates them from the maternal blood spaces , but which in certain areas is deficient , so that the endothelium is all that separates the foetal from the maternal blood. The mesoderm is thin and loose in structure , and contains no fibrinous deposit.

Punctured vaginal Sac of Left Horn - removed post mortem.

The wall is 1.5 mm. in thickness at the placental site. The muscular coats are dense and compressed , there being very little cellular tissue between them. There is a great increase in the quantity of sub-mucous tissue, which is of a very loose structure and extremely vascular. The surface epithelium is undergoing active proliferation , so that it is in places plasmodial , and there is also glandular hypertrophy. The normal mucosal folds are beginning to make their appearance , and in them the epithelial proliferation is most marked. There are no giant cells present.

Maternal Placenta :- The zone of separation is narrower and the tissue more reticulated than in the normal 27 days sac , this being due to the greater shrinking of the cells (fig. 16). There is no fibrinous deposit between the cells. The rest of the maternal placenta consists of fibrin and coagulated

lymph , enclosing in its meshes the degenerated remains of decidal cells , leucocytes and multinucleated giant cells. Only one or two islands of decidual cells remain. These surround blood sinuses with fibrin walls , from which fibrin threads extend out among the cells. The sinuses are large and approach up to the face of the foetal placenta , as do those in the normal 27 days sac , and in contrast to those in the 16 days placenta. The fibrinous deposit is much more extensive than in the normal 27 days sac , and the number of patent sinuses fewer. Many of the larger ones are filled with coagulated blood.

Foetal Placenta :- This shows more marked degenerative changes than have hitherto been seen in any of the cases (fig. 27). The whole area is extensively invaded by fibrin and coagulated lymph , and even microscopically the distinction between maternal and foetal portions is not clearly marked. The ectoderm for the most part is represented by a homogeneous , purple staining mass , no cell outlines or nuclei being visible , and no division into columns. Blood vessels in these areas are entirely absent. Nearer the foetal surface faint outlines of the original structure can still be detected , the faintly staining protoplasm of the ectoderm being seen separating blood vessels filled with degenerated or " ghost " corpuscles, while scattered throughout

are interlacing threads of fibrin and cell debris. No nuclear staining is present in this ectoderm , but towards the maternal surface it is still retained. Even in the latter area the ectoderm is broken up by fibrin laminae , and the vessels which it surrounds contain only degenerated corpuscles and debris. The mesoderm at the surface , and where visible in other parts of the placenta , consists almost entirely of fibrin and coagulated lymph , the mesoderm cells being few and scattered.

Foetus :- The epithelial covering is completely shed. As in the other degenerated foetuses the connective tissue cells are numerous , are closely arranged , and have large swollen granular nuclei. In the present case this degenerative change does not seem to be more advanced than that present in Experiment III. The ground substance is composed of coagulated lymph and some debris. There are a few multinucleated giant cells present. No blood vessels or corpuscles are seen. Cartilage formation has proceeded only to the extent seen in the 15 days foetus , and the cells composing it show very little degenerative change compared with the ordinary connective tissue ones , still the nuclei are distinctly granular.

The heart is represented by a few scattered fibres entirely detached from each other in the midst of a collection of

round granular cells. The protoplasm of the fibres is broken up and nuclear staining is entirely wanting (fig. 34). No endothelial lining is visible , and indeed the cavity of the organ cannot be recognised.

The liver is with great difficulty recognised at all , and consists merely of some granular debris and connective tissue cells with round granular nuclei , among which are embedded a few irregularly shaped liver cells , the protoplasm of which stains very feebly , and which show no nuclear staining at all. No blood corpuscles are anywhere visible.

The kidney as in the other experiments is not degenerated to the extent that the other organs are , the characteristic structure of the organ still being easily recognisable. It shows the same stage of development as in the 15 days one. The glomeruli are obscured by round cells with granular nuclei; they are compressed and thickened and contain no blood (fig. 41). The epithelium of the tubules is swollen and the protoplasm is breaking down at the periphery of the cells. The nuclei are very granular and stain feebly. In many of the tubules the epithelium is entirely absent , and in others it is seen in the process of detachment , the complete lining being thrown off into the lumina (fig. 41). Between the tubules and the glomeruli the connective tissue cells are extensively degenerated.

The pancreatic gland tubules show less change than any of the organs. The only degeneration present is a slight swelling of the columnar epithelium and granulation of the nuclei.

Membranes :- The yolk sac is shrivelled and in close contact with the amnion and foetus. The mesoderm is occupied by fibrin , lymph and cell debris. The cells are rounded and have granular swollen nuclei. Blood corpuscles are here and there visible with shrunken bodies and no nuclei. The epithelium is mostly shed from the surface , but here and there large giant buds of multinucleated protoplasm have been formed by the coalescence of neighbouring cells. Many of these buds have become entirely detached. The amnion closely invests the foetus.

Punctured tubal Sac from Left Horn - removed post mortem.

The microscopic appearances in this are exactly the same as just described in the other punctured sac , and to describe them would be to repeat what is said above. The foetus is flattened out in the same way , and shows the same degenerative changes.

SUMMARY OF THE RESULTS OF EXPERIMENT VII.

There has been no re-secretion of the fluid. The pressure of the uterine walls has compressed the placenta from side to side , thus increasing its vertical depth. The foetus has died , and become flattened out between the surface of the placenta and the roof of the sac. The amniotic mambrane is closely applied to it all round so that there is no fluid in it. The vascular yolk sac wall is still present. The changes in the maternal placenta are of the same nature as those previously noted , while the foetal placenta shows a very extensive invasion of fibrin , both in the mesoderm and in the ectoderm. In addition , the ectoderm is very much more extensively degenerated than in the punctured sacs of the previous experiments.

EXPERIMENT VIII.

Operation when 12 days pregnant.

Animal killed when 26 days pregnant.

Interval = 14 days.

Operation :- On opening the abdomen there were found to be five sacs in the right horn , and two in the left. The tubal sac of the right horn was punctured , and 2 cc. of slightly turbid fluid withdrawn. No sac was excised. The animal recovered perfectly , and was killed 14 days later, it then being 26 days pregnant.

On post mortem examination the abdomen was found to be healthy. There were no adhesions. In the left horn of the uterus were two large sacs , and in the right , three sacs of similar size. The terminal tubal sac which had been aspirated was very small - only a little larger than at the time of operation , and it was firm in consistence , apparently containing no fluid. The sac next it was slightly smaller than the others , but contained abundance of fluid.

DETAILED DESCRIPTION OF THE SACS.

1. Naked eye Appearances.

Normal 12 days Sac.

For a description of this see Experi-

ment II.

Normal Sac from Right Horn - removed post mortem.

The transverse section is ovoid in shape , measuring 3.3 cm. in vertical by 3.1 cm. in transverse diameter. The placenta is .9 cm. in depth , of which .6 cm. is foetal , and .3 cm. maternal. The space between the placenta and the roof of the sac is 2.3 cm. There were 4.1 cc. of amniotic fluid. The foetus is 7 cm. in length. The maternal placenta is only half the depth of the foetal , and is pale in colour with a few haemorrhagic areas. The zone of separation stands out as a clear translucent band next the uterine wall. The foetal placenta is of a dark red colour. There are no haemorrhages in it.

Punctured Sac from Right Horn - removed post mortem.

The sac is almost circular in transverse section , measuring 1.5 cm. in vertical by 1.3 cm. in transverse diameter. The placenta is 1 cm. in depth , of which .6 cm. is maternal , and .4 cm. foetal apparently , but the distinction between the two is very difficult to make out. The space between the surface of the placenta and the roof of the sac is .3 cm. , and in this is what remains of the foetus - a small grey body which is only .2 cm. in transverse section. The zone of separation in the placenta is well marked , and the rest of it is of a greyish colour

with numerous , small , redder areas throughout. Towards the surface the colour approaches more to brown , and this is the part which is regarded as the foetal placenta. There was no fluid at all in the sac.

2. Microscopic Examination.

Normal 12 days Sac.

For a description of the microscopic appearances , see Experiment II.

Normal Sac from Right Horn - removed post mortem.

The wall is .5 mm. in thickness. The muscular layers are separated by loose cellular tissue , containing large vessels. The mucosa is flattened , and no giant cells are present.

Maternal Placenta :- The zone of separation is made up of shrunken decidual cells which form a loose reticulum. The vessels in it are small and have slightly swollen endothelial walls. The rest of the placenta is composed for the most part of fibrin tissue and debris. In it are dilated sinuses containing blood. Many of them are thrombosed. Those that are still patent are lined by thick fibrin laminae , and in some cases the cast off swollen endothelial lining is still present in the lumen. Round the vessels some multinucleated decidual cells are still distinguishable among the fibrin

tissue. Further away from the vessels other cellular elements can be seen - large multinucleated giant cells and leucocytes , and the remains of decidual cells.

Foetal Placenta :- This consists of foetal and maternal vessels arranged in columns with a very thin layer of plasmodial ectoderm separating them. There is no fibrinous deposit either in the surface mesoderm , or in the region of the ectodermic columns , and nowhere does the ectoderm show any sign of degeneration.

The foetus is now practically fully formed with all the organs as they are at full time.

Punctured Sac from Right Horn - removed post mortem.

The wall is 1 mm. in thickness and the muscular layers are in close apposition. There is some hypertrophy of the sub-mucous tissue resulting in a restoration of the villous processes of the mucosa. No giant cells are present.

Maternal Placenta :- The zone of separation is made up of an even looser tissue than the normal , as the decidual cells are more shrunken. A few fine fibrin threads are present between the cells. The rest of the placenta is made up of fibrin tissue and debris. The great majority of the vessels are completely fibrosed , and those that are patent are lined by fibrin laminae. The cellular elements in the

fibrin tissue are much more scanty than in the normal placenta. No decidual cells are visible in the greater part of the area, and it is only as the region of the foetal placenta is approached that they become distinguishable , and that only with difficulty owing to their faint staining. The fibrin laminae are denser than normal.

Foetal Placenta :- This is only with difficulty recognised as such. At the surface the mesoderm is densely invaded by thick fibrin laminae (fig. 28) and cell debris. These extend everywhere throughout the foetal placenta , so that , as in the maternal , they make up the bulk of the tissue. In the greater extent no trace of the original structure is visible , but in one or two areas which are more numerous towards the maternal surface than elsewhere , indications of the presence of ectoderm and its arrangement round the vessel walls are visible. The vessels are filled with " ghost " corpuscles - no normal red blood corpuscles are anywhere visible in the foetal placenta - and round them is a faintly layer of protoplasm with , at long intervals , blue staining nuclei. That is all that remains of the ectoderm. In the surface mesoderm no vessels can be seen.

Foetus :- The epithelial covering is entirely lost. The connective tissue cells are more degenerated than in any of the other cases. Most of the nuclei are represented by a

collection of faintly staining granules , while the protoplasm of the cell body has in many cases entirely broken down. There are many giant cells present , apparently forced by the coalescence of neighbouring cells. There is no blood seen in any part. There is no cartilage formed.

The heart is scarcely recognisable , and is represented by a few scattered muscle fibres which stain very feebly and irregularly with eosine , and which have lost their nuclei. Between these are many round cells.

No trace of the liver can be detected.

The kidney shows more desquamation of the tubules than in the last cases. The nuclei still stain fairly well , but the protoplasm of the cell bodies is more broken down. The glomeruli have the same appearance as in Experiment VII.

Membranes:- The mesoderm of the yolk sac wall is infiltrated with fibrin and the debris of broken down cells. The cells that remain are degenerated. There are no blood corpuscles visible. The epithelium is lost except in places where it has become plasmodial and has budded off from the wall.

The amnion shows no change.

SUMMARY OF THE RESULTS OF EXPERIMENT VIII.

There has been no re-secretion of the fluid. The foetus has died , and the placenta has undergone the same series of changes as have been noted in the preceding experiments. The maternal placenta has advanced in development from the condition found in the 12 days sac to that which obtains about full time - it has been accelerated in its development. The foetal placenta on the other hand , has undergone changes which never occur during normal gestation. There has been a very extensive deposit of fibrin in all parts , beginning in the mesoderm. This has compressed the ectoderm and it has degenerated to such an extent as almost to have completely disappeared. The foetus has undergone the same series of changes as have been noted in the others.

SUMMARY AND DISCUSSION OF RESULTS

SUMMARY AND DISCUSSION OF RESULTS.

The following table shows the intervals of time which have been allowed to elapse between the operation and the death of the animal in the several experiments.

Experiment I.

Animal 16 days pregnant.

Interval between operation and death - 3 days.

Experiment II.

Animal 12 days pregnant.

Interval between operation and death - 5 days.

Experiment III.

Animal 14 days pregnant.

Interval between operation and death - 7 days.

Experiment IV.

Animal 21 days pregnant.

Interval between operation and death - 7 days.

Experiment V.

Animal 16 days pregnant.

Interval between operation and death - 9 days.

Experiment VI.

Animal 10 days pregnant.

Interval between operation and death - 10 days.

Experiment VII.

Animal 15 days pregnant.

Interval between operation and death - 12 days.

Experiment VIII.

Animal 12 days pregnant.

Interval between operation and death - 14 days.

In addition in Experiment I one of the punctured sacs was removed at the operation after an interval of 10 minutes.

The following table shows the stage of gestation at which the different experiments have been performed.

Experiment VI	at the	10th day.
Experiments II and VIII	" "	12th day.
Experiment III	" "	14th day.
Experiment VII	" "	15th day.
Experiments I and V	" "	16th day.
Experiment IV	" "	21st day.

It is unfortunate that it was impossible , owing to the difficulty of breeding rabbits in the winter months , to obtain sufficient material to carry out a larger number of experiments at the same stage of gestation , and note the changes resulting after the lapse of varying intervals of time in each. I thought it more satisfactory to vary the time of operation in such animals as I was able to obtain , and so cover a greater amount of ground , than to operate on them all at the same stage of gestation , and this has been justified , as the changes induced have all been of the same nature , no matter what the stage of pregnancy has been at the time of operation. It will be seen from the above tables however , that two animals were operated on at the 12th day , and two at the 16th; only one was used at each of the other stages.

In Experiments II and VIII at the 12th day , and in Experiment VI at the 10th day the fluid withdrawn was the yolk sac fluid , while in the other five animals the amniotic fluid was aspirated. The reason for this is , as explained in the sketch of the development of the foetal membranes , that the amnion is not completely closed in until the 10th day , and is not distended with fluid till after the 12th (fig.1).

The results obtained by the withdrawal of the yolk sac fluid in no way differ from those obtained by the aspiration of the liquor amnii , the probable explanation of this being that in both cases the first effect is to cause the death of the foetus , and that what follows is the consequence of this. For we have noted in all the experiments that the foetuses of the aspirated sacs show no advance in development from that to which they had attained at the time of operation , and that they had undergone degenerative changes (figs. 5 and 10).

The sequence of events after the withdrawal of the fluid, be it yolk sac fluid or liquor amnii , then , is :-

1. Death of the foetus and subsequent degeneration.
2. Arrested secretion of the liquor amnii.
3. Degeneration of the foetal placenta.

These three results will be dealt with separately in what follows , the discussion on the liquor amnii being taken up

after the other two have been dealt with.

THE FOETUS.

All the experiments have resulted in the death of the foetus , whether the fluid withdrawn was the liquor amnii , as in Experiments I , III , IV , V , and VII , or the yolk sac fluid , as in Experiments II and VIII; (in Experiment VI all foetal structures had disappeared). Further , death must have occurred very soon after the operation as in all the cases the size and development of the foetus corresponded with that of the one in the normal sac excised at the operation.

Cause of Foetal Death.

Since foetal death occurred in all the cases , and that apparently in the same way , it follows that the cause is something other than the mere withdrawal of liquor amnii , for as mentioned above , in Experiments II and VIII the latter was not interfered with.

Great care was in all cases exercised to avoid direct injury to the embryo by the aspirating needle , and I am convinced that in no instance was any such injury inflicted , for had the foetus been punctured I should have easily recognised the fact at the time , and at the subsequent post mortem examination signs of it would have been present , and in no case was this so.

It may be that in the two sets of cases - the aspiration of the yolk sac fluid and the aspiration of the liquor amnii - the cause of foetal death has not been the same. Robinson, from a histological examination , concludes that in rats and mice the yolk sac is an important nutritive organ , and that up to the 11th day it is probably the only source from which the growing embryo derives nourishment , and that right up to the end of gestation it plays a certain part in supplying that nourishment , although in the later weeks , after the allantoic circulation is established , it is only a secondary one. Now although the arrangement of the yolk sac in the rabbit almost certainly points to it taking a smaller share in the nutrition of the embryo than does that of the rat or mouse , still it must be of some nutritive or respiratory importance for the circulation in it , while attaining its maximum about the 10th or 11th day , persists right up to the end of gestation. In the early days the outer wall is in direct contact with the inner wall of the uterus , and is there anchored by epithelial buds , and it is probable that nutritive material is thus passed into the yolk cavity and thence absorbed by the blood in the vascular part of the wall , and so carried to the embryo (fig. 1). After the 13th day when the outer wall is absorbed and the cavity ceases to exist , the vascular wall itself comes into contact with

the uterine mucosa , and the same process may go on directly without the material passing through the yolk sac cavity at all (fig. 2).

Any nutritive function the yolk sac may have at the stages of gestation at which these experiments have been performed must be a very secondary one as compared with that carried on by the allantoic and placental circulation. Still the interference with the vitelline circulation consequent on the collapse of the wall after the aspiration of the fluid , as in Experiments II and VIII , may be sufficient to lead to the immediate death of the embryo. There have been noted in all the cases very extensive changes in the vascular wall of the yolk sac , consequent on the aspiration of the fluid. There has been a leakage from its vessels resulting in a lymph and fibrin deposit in the mesodermic tissue. The blood corpuscles have degenerated , and all circulation has ceased , while the epithelial covering has degenerated. There is , however , no direct evidence to decide as to whether these changes are the cause or the result of foetal death.

The possibility must also be kept in mind that in these two cases operated on at the 12th day , the extra-embryonic coelom may have been entered by the needle , and some fluid withdrawn from it , resulting in the death of the foetus. That this is possible is shown by fig. 1

In the cases in which the amniotic fluid was aspirated there is also no direct evidence as to the cause of foetal death. From the very slight changes noted in the placenta for the first few days after operation , as in Experiment I , compared with the extensive alterations which the foetus has undergone , it is certain that the foetal death is not due to any degenerative change in it. It was noted in the sac which was examined 10 minutes after aspiration in Experiment I that the uterine wall had contracted so as to follow up the collapsed amniotic sac , and that this contraction had resulted in the compression of the placenta from side to side , so that the ectodermic columns were more closely compressed , and that in consequence the foetal vessels in the mesoderm were partly emptied of their blood and had their walls approximated. This compression of the placenta has been a constant feature in all the punctured sacs , as has also the compression and obliteration of the foetal vessels in the mesoderm. The muscular contraction has in no case however, been of sufficient strength to compress the placenta to such an extent as of itself to arrest the circulation in it completely , for in all there are numerous bands of mesoblast running throughout the foetal placenta , and in these the vessels are not compressed.

The explanation of the death of the foetus is rather to

be found in the arrest of the circulation consequent upon the general alteration of pressure within the sac resulting from the withdrawal of fluid , this arrest taking place both in the allantoic and vitelline system of vessels. The latter will be more liable to easy derangement because of the unsupported nature of the attenuated wall of the yolk sac , and I think it very probable that , as in the cases where the yolk sac fluid was withdrawn , so in these in which the liquor amnii was aspirated , the interference with the vitelline circulation consequent on the collapse of the yolk sac plays a very important part in causing the death of the foetus.

The mere absence of amniotic fluid may play a part , but on this point there is no direct evidence to adduce. That the liquor amnii is not in all cases necessary for the life of the foetus is shown by cases of extramembranous development of the human foetus , such as have been recently recorded by Maygrier, Cathola and Perret.(1)

Degenerative Changes.

The changes which the foetus has undergone after its death have been the same in all cases. The naked eye appearances have been very characteristic. The normal pink , translucent appearance of the living foetus has given place to the dull grey aspect of the degenerated one , while the rounded contour has been entirely lost , and the dead

(1) In these cases the amniotic sac was represented by a small pouch situated on the surface of the placenta close to the insertion of the cord , while the foetus was completely outside it,but still alive at , or shortly before,the time of birth.

foetus has become flattened out between the face of the foetal placenta and the upper wall of the uterine horn in which it lies.

In its flattened appearance which is well shown at an early stage in fig. 5 , and at a later stage in transverse section in figs. 2 and 12 , it resembles the foetus papyraceus of occasional occurrence in twin pregnancy in the human subject. In this connection it is interesting to note that no case of foetus compressus has been known in the human subject in any other than a twin pregnancy , where the pressure of one of the foetuses flattens the other. In only one instance in these experiments (Experiment IV) has the flattening been produced by the pressure of the other foetuses in the cornu; in all the other cases it has been due to the pressure of the uterine wall itself against the face of the placenta.

The exact significance of the pathological appearances of the foetus papyraceus is a disputed one. Ahlfeld and Schatz hold that the flattening is an essential teratological condition. The latter , from a study of 7 cases , comes to the conclusion that as a result of the imperfect blood supply to one of the foetuses in some cases of uniovular twin pregnancy the nutrition of that twin suffers and there is oligohydramnios. This , together with the thinness of the foetus,allows of the latter being readily compressed.

Ballantyne , on the other hand , regards the flattening as of secondary occurrence following the death of the foetus from such causes as insufficient placental circulation in the common placenta , or from placental haemorrhage , and subsequent absorption of the liquor amnii. My results entirely bear out this latter view , viz. , that the first thing to occur is the death of the foetus , and that , after softening and degeneration have begun , it becomes flattened out. This after all , is what one would expect , for the natural resilience and elasticity of the living tissues are very great and are sufficient to prevent any marked deformity , which , on the other hand , is readily produced when these are lost as a result of death and degenerative changes.

In none of the cases has there been swelling of the tissues and accumulation of sanguinolent fluid in the abdomen such as is met with in macerated foetuses which have remained in the human uterus bathed in liquor amnii for some time after their death. Hourlier , who has examined a number of such cases , explains the accumulation of fluid as resulting from the breaking down of blood clot which has formed in the pleural and peritoneal cavities as the result of the death of the foetus from asphyxia. There have been no such haemorrhages in any of my cases.

By microscopic examination a progressive series of degen-

erative changes can be traced in the various tissues and organs as the time of retention in the uterus lengthens from 3 up to 14 days , as in the several experiments. Experiment IV , in which the death of the foetus occurred at a later stage in its development than in any of the other cases , forms a slight break in this progressive sequence , as the changes noted in it are not so advanced as in the other case where the interval of time between the death of the foetus and the death of the mother was the same , viz. , 7 days.

The degenerative process begins very early in all the tissues , but the rate at which it advances varies considerably in the different organs and histological elements.

The surface epithelium is very soon entirely lost when the death of the foetus occurs at any time in its development up to the 16th day. Thus three days after death , as in Experiment I , all trace of it has been lost. On the other hand , in Experiment IV , where the foetus was 21 days old when it died , the epithelium is still in places intact , after an interval of seven days. In this latter case the process by which the shedding of the epithelium is effected can be traced. The individual cells undergo a process of breaking down , so that the protoplasm swells and disintegrates at the cell margins , while the nuclei break up into a mass of granules. In this way the attachment to the subjacent

structures is loosened , and large areas become detached and thrown off.

The connective tissue cells are early affected (fig. 30). They undergo a process of granulation and breaking down. The nuclei become swollen , rounded and granular , while the protoplasm in the periphery of the cells , and especially in their branching processes , breaks down , so that the cells become rounded in shape. The ground substance becomes infiltrated with the debris of broken down protoplasm , and a substance like coagulated lymph. All the changes are already well advanced by the 3rd day , and from this time up to the 12th , very little further degeneration can be detected. After the latter interval the nuclei are still more granular , and the granules are finer , but in only a few cells have they disappeared entirely. These changes are equally marked in the connective tissue of the body wall , and that forming the frame work of the various organs.

In only one case was cartilage fully formed before the death of the foetus. The degenerative changes which it has undergone after an interval of 7 days are comparatively slight. There is absolutely no change in the ground substance , but the cells have shrivelled and shrunk , and the nuclear staining is faint and irregular (fig. 42).

The liver and the heart are the organs which undergo most rapid degeneration and disintegration. After an interval of 12 days retention in the uterus they are scarcely recognisable. The kidney and pancreas on the other hand , break down much more slowly.

After an interval of 3 days the liver is extensively degenerated. The process , as in the connective tissue cells , is one of swelling and disintegration of the protoplasm of the liver cells , and granulation of the nuclei. The cell outlines early become lost , and the nuclear staining faint , and after that the cells break down completely , and nothing remains but a mass of debris , with an occasional faintly staining protoplasmic mass which has something of the shape of the normal liver cell (figs. 35-38.) This is the condition of the liver in Experiment VII , after the lapse of 12 days since the death of the foetus. The blood in the liver , which in the normal organ is very abundant , very early begins to break down. The delicate vessel walls give way , and the corpuscles lie free among the debris of the broken down liver cells , where they become shrivelled , lose their nuclear staining , and finally completely disappear.

The muscle fibres of the heart walls undergo an analogous process of breaking down. The endothelial lining becomes detached and is cast off in large areas and thrown into the

cavity. The fibres lose their striation and become broken up longitudinally , while thenuclear staining becomes faint and granular. As the interval lengthens from 3 to 5 and so on up to 14 days , these changes become more and more marked , and the protoplasm begins to break down completely , and forms a mass of debris in which only a few fibres can be distinguished. The heart wall thus comes to consist of swollen and degenerated connective tissue cells (which resist longer than the more specialised heart fibres) embedded in a ground work consisting of protoplasmic debris and extravasated lymph (figs. 31-34).

The ordinary striped muscle of the trunk and limbs undergoes a similar degenerative process to that of the heart. The fibres become split up longitudinally into their individual fibrils , while the nuclei swell up , become granular and disappear (fig. 42).

The kidney tissues resist much longer than those of the liver. The cells of the tubules become swollen with faintly staining and granular nuclei , but the extent of the degeneration is always less than in the liver of the same animal , although the process is exactly of the same nature , viz. , a sort of cloudy swelling , followed by disintegration of the cell protoplasm beginning at the periphery , together with a breaking down of the nuclei. In the case of the

kidney , however , the epithelium of the tubules is in many cases quite intact after an interval of 12 days , at which time , and in the same animal the liver can scarcely be distinguished. As a result of the disintegration of the peripheral protoplasm of the cells of the tubules the lumina become filled with debris , and ultimately the whole epithelial lining becomes detached (figs. 39-41).

The glomeruli first shrink and the blood in them breaks down , and then the epithelium of the capsule degenerates , so that after an interval of 12 days they come to look like dense aggregations of degenerated connective tissue cells , in which no definite glomerular structure can be distinguished.

The cells of the pancreas show less departure from normal than any. They can evidently resist the process of disintegration better than the others. It is a noticeable feature in my sections that the pancreas in all cases stains a very deep blue with haematoxylin , which probably indicates that it is of a strong alkaline reaction. This may have something to do with its resisting powers. Even after an interval of 12 days all the degenerative process that cen be detected in it is a slight swelling of the cell bodies and faint granulation of the nuclei.

The epithelium of the stomach and intestine undergoes the same degenerative process as that noted in the kidney and

liver , and ultimately it becomes completely detached and thrown into thelumen. The wall of the stomach , owing to the swelling of the connective tissue cells and the degeneration and disappearance of the other elements , looks as if it were entirely composed of the former.

The degenerative change which all the tissues of the retained dead foetus undergoes is therefore of the same character , and is of the nature of a coagulation necrosis. That is , the protoplasm of the cellular elements breaks down and disintegrates , while the debris so resulting accumulates and becomes mixed with extravasated and degenerated blood , giving the appearance of fibrin formation.

The tissues which resist longest are the least highly specialised , namely , connective tissue and cartilage , while those that disintegrate soonest and most completely are those of the liver. The degeneration of the kidney elements is a slower process while the pancreas is still in a comparatively normal state when the former is extensively altered.

In none of the organs or tissues is there any evidence of any proliferation of the cellular elements after the death of the foetus as a whole , and the apparent increase in the connective tissue cells is due to the earlier and more raped degeneration of the other elements and the compression of the foetus.

THE UTERINE WALL AND PLACENTA.

Uterine Wall.

As the result of the withdrawal of the fluid from the sac the uterine wall very soon contracts , so as to compensate for the loss and counteract the negative pressure inside. This it does as shown in the punctured sacs excised 10 minutes after aspiration in Experiment I, by the contraction and thickening of both the inner circular and the outer longitudinal muscular layers. This contraction is quite local and confined to the area of the sac ; no such thickening of the wall of the unpunctured sacs excised at operation has been noted. The contraction never attains such a degree as to render the muscular wall of the sac as thick as that of the non-pregnant uterus , but that it is fairly active is shown by the compression from side to side of the placenta , so that the two lobes become more closely approximated. This was noted in the sac removed 10 minutes after puncture , and is a characteristic feature in the placentae of the punctured sacs in all the other experiments. Besides causing approximation of the two halves of the placenta this muscular contraction also compresses individual elements leading to a condensation of the tissue. By counteracting the negative pressure inside the sac it is doubtless

an important factor in preventing haemorrhage into it , and into the placenta immediately after aspiration. It also results in compression of the blood vessels between the two muscular layers , and so diminishes the vascular supply to the placenta.

The folds of the mucous membrane which had been obliterated by the distension caused by the growing ovum are again reproduced to some extent when the distension is relieved , and during the remainder of the time the animal is allowed to live the sub-mucous tissue hypertrophies , and the surface and glandular epithelium proliferates , so that the mucosa approaches in appearance to that of the non-pregnant uterus. No marked changes in the production and degeneration of the giant cells of the mucosa has been observed.

Placenta.

The only changes observed in the sac excised 10 minutes after aspiration in Experiment I are the compression of the two lobes of the placenta from side to side , and the pressing together of the columns of ectoderm in the foetal placenta so that the mesoderm with its contained vessels is compressed. This is the result of the contraction of the muscular layers of the sac.

After an interval of 3 days , as shown in Experimnt I ,

the walls of thepunctured sacs are further thickened , owing to muscular contraction , and this has resulted in more marked compression of the placenta from side to side , so that its vertical depth is increased , and it comes to occupy a much larger vertical area of the sac than normal. At the same time the naked eye appearances of the placenta differ from normal , owing to the diminished vascularity of the foetal portion due to the compression of the ectodermic columns and the flattening of the vessels in the mesoderm. There is a slight deposit of coagulated lymph in the mesoderm at the surface and in the substance of the foetal placenta. In one or two isolated areas the ectoderm shows signs of commencing degeneration as evidenced by the deficient nuclear staining , but it still retains the power of penetrating the maternal tissues. The maternal placenta differs from the normal 19 days one only in the greater amount of fibrinous deposit among the decidual cells , and the more distinct differentiation of the zone of separation , partly due to this, and partly to the greater attenuation of the tissue itself.

The placenta as a whole therefore , 3 days after the death of the foetus shows only slight departures from normal. It has gone on developing along the normal lines from the 16th to the 19th day , and in the case of the maternal part shows an accelerated development by the more extensive fibrinous

deposit and the more marked zone of separation , so that it has the appearance of a 21st or 22nd day placenta rather than a 19 days one.

In Experiment II 5 days were allowed to elapse between the operation and the death of the animal , 48 hours longer than in Experiment I. During that time very marked changes have occurred. As before , the thickening of the uterine wall and compression of the placenta are marked features, while to the naked eye the distinction between the maternal and foetal portions is less obvious , owing to the diminished vascularity of the latter. The maternal part shows more fibrinous deposit and a more marked zone of separation , as compared with the normal than is present in Experiment I , while many of the vessels are fibrosed.

The foetal placenta now shows very marked signs of degeneration. The mesoderm is more extensively invaded by threads of fibrin which in places form laminae. The ectoderm in the centre is extensively degenerated , nuclear staining being lost and the protoplasmic staining irregular. At the maternal surface , while the degeneration is less marked , the power of actively penetrating the maternal tissues has been lost.

After an interval of 7 days , as in Experiments III and IV all these changes are more marked. As the result of the

accelerated development of the maternal placenta in Experiment IV , the placenta has become detached 2 days before full time , while in Experiment III it shows more fibrinous deposit and a more attenuated zone of separation than in the previous cases. The foetal ectoderm is further degenerated , so that in the central areas its structure is altogether lost. The mesoderm shows increased fibrinous deposit , and the ectoderm where distinguishable , has in most places lost its nuclear staining , and the protoplasm is granular.

As the interval that is allowed to elapse between the operation and the death of the animal lengthens from 7 to 9 , 10 , 12 and 14 days as in the remaining experiments , a progressive series of changes can be traced along the lines sketched above. The fibrinous deposit in the maternal placenta becomes more and more extensive and the fibrin alminae become denser , so that the decidual cells are compressed, and degenerate and disappear. The fibrin laminae round the vessels becomes thickened to such an extent as to obliterate the vessels , many of which thus become entirely fibrosed. The tissue forming the zone of separation becomes more and more attenuated as contrasted with the normal. The fibrinous deposit in the foetal mesoderm becomes more marked , and after an interval of 9 days begins to invade the ectoderm , breaking it up and compressing it. The ectoderm undergoes a pro-

gressive degeneration , and ultimately almost completely disappears among the mass of fibrin which after an interval of 14 days comes to make up the great part of what ought to have been the foetal placenta.

It is thus evident that the changes which result in the placenta following on the death of the foetus are of an entirely different nature in the two component parts - maternal and foetal. The former goes on evolving along the normal lines , and all the departures from normal are merely of degree and are an expression of accelerated development. The foetal placenta on the other hand undergoes processes which never occur in the normal sac , such as theinvasion of the mesoderm by coagulated lymph and fibrin tissue and the degeneration of the ectoderm.

The fibrinous deposit which occurs in the maternal portion takes place in the same situations as in the normal sacs, and there is no evidence to show that its mode of production differs in any way from normal , viz. , by a slow leakage through the fibrin lined walls of the maternal sinuses. The only difference is that it occurs more quickly and more extensively , so that a placenta which is at , say , the 20th day , presents appearances like those met with at the 22nd or 23rd day. This behaviour of the maternal placenta I think ,

throws considerable light on the rarity with which abortion was induced during the course of this investigation. It is a remarkable fact that such interference as the uterus was subjected to in every case - the manipulation , the aspiration of fluids from the sacs , and the complete excision of part of the cornu , resulted in abortion in only one instance , and in that case the animal was at the 26th day of pregnancy. When first the idea of carrying out this work was suggested to me the probability of producing abortion almost acted as a deterrent , and the first few animals operated on were anxiously watched for its occurrence , but as mentioned above, it only happened once.

I have not been able to find in any literature on the rabbit's placenta whether abortion is a frequent occurrence or not; that it does occur occasionally is very probable. That abortion is prevented by the presence of other sacs on the proximal side of the punctured one is negatived by the fact that even when there is only one sac in the horn , and that is aspirated , as in Experiment III , it did not occur.

From the structure and mode of development of the maternal placenta we should not expect it to be easily cast off in the early stages before the zone of separation has been formed. In its early stages it is practically one with the with the rest of the uterine cornu , possessing a broad base

of attachment , and being composed of dense well formed tissue. The subsequent stages which it goes through are for the purpose , firstly , of allowing a sufficient blood supply into which the foetal structures may dip , and providing a tissue , fibrin , through which they can readily penetrate; and secondly , of providing a zone through which the whole may be cast off with the minimum loss of blood and destruction of tissue.

The latter phase of development has been accelerated and exaggerated in all the placentae of the punctured sacs , for in all of them we have noted a development of the zone of separation , and an attenuation of its cellular elements in advance of the normal at the period examined. This I take to indicate a tendency to the earlier casting off of the dead ovum , and in Experiment IV this had actually taken place , the whole ovum and maternal placenta lying free in the uterine cornu , the latter having been detached through the zone of separation at the 28th day , while the normal placentae were quite firmly attached and would presumably have remained so for two days longer when full time would have been reached.

It is a well known fact that it is often extremely difficult to induce abortion by artificial means in the human female , provided that both she and the ovum be healthy , and that in many cases nothing short of actual manual detachment

will serve. This is due to the close incorporation of foetal and maternal structures , and to the fact that " zone of separation " has not developed. In the placenta of the rabbit we see the development of this zone much more clearly and definitely than in that of the human subject , and its accelerated development in the cases where the foetus is dead is , I think , extremely instructive , as showing that while the tendency is to abortion , this cannot take place until a certain definite series of changes have been gone through. What it is that determines the formation of the zone of separation in the placenta is not known , but whatever it is these experiments show that the cause cannot be dependent in any way upon the vitality of the foetus belonging to it. Indeed one of the most remarkable things about the whole series of these experiments is the demonstration of how little the development of the maternal placenta is so dependent. It has been a much disputed point as to what factors determine the onset of labour. These experiments prove that one essential condition , viz. , the preparation of the placenta for ready detachment , does not depend on foetal but on maternal influences.

I would here then , emphasize the fact that the dead and degenerated ovum and placenta are not cast off till certain definite developmental changes have been gone through , which

are in every respect analogous to those which determine the separation of the normal placenta.

What the behaviour of the placenta would be when the initial cause of its degeneration resided , not in the foetal but in the maternal structures , I have not determined. It is conceivable that a large haemorrhage , for instance , occurring in the substance of the placenta close to the line of attachment to the uterine wall , would so tear up the tissues as to completely detach the structure before the normal zone of separation had developed sufficiently to enable this to occur naturally. It is known that abortion in the human subject is sometimes due to such a cause , and that the placenta quickly becomes detached , and the ovum is almost immediately expelled in a perfectly fresh condition with a foetus which has the normal appearances that obtain at the period of pregnancy at which the abortion occurred , and which may still be alive at the time of expulsion.

On the other hand it is common to find in examining an abortion sac that the placenta and decidua present appearances which differ little from normal , while the foetus is in an advanced state of softening and disintegration , and has evidently been dead for some time. It has accordingly been inferred that such cases of abortion are due primarily to the death of the foetus , and that the ovum has been re-

tained for some time thereafter , and that the placenta has undergone few pathological changes.

Referring to this subject Ballantyne makes the following observations. " It is quite possible that there may be a much greater independence between the vitality of the foetus and that of its annex than has hitherto been supposed , and that , when the cause of foetal death resides in the foetus itself , the life of the placenta may to a certain extent be continued. When , on the other hand , the foetus dies because the placenta is practically dead , the dependence will be more manifest ....." " When the foetus has been dead and markedly macerated I have found villi in the placenta containing apparently normal blood cells , and I do not regard it as impossible that the placenta may increase somewhat in size after the decease of the unborn infant. "

No perfect analogy can of course be drawn between the behaviour of the placenta of the rabbit and that of the human female , but the broad general lines of their structure and development are essentially the same , and the degree of dependence of the foetal on the maternal structures and vice versa must be identical , so that I think we are justified in regarding the sequence of events as they occur in the rabbit's placenta following on the death of the foetus , to be the same as that which occurs in the human placenta under

similar circumstances. So that judging from the results which I have obtained in the rabbit , the explanation of these cases of abortion in the human female which result in the expulsion of a little altered sac and placenta , but with an extensively degenerated foetus , is to be found in the fact that the initial cause of the abortion has been the death of the foetus and not any morbid process in the placenta itself.

The changes which occur in the foetal placenta are of a more distinctly pathological nature than those which occur in the maternal , and are mostly an expression of the death of the tissue. This death however , does not occur for some time after the death of the foetus , for after an interval of three days as in Experiment I , the changes observed were only slight , and there was distinct evidence that the placenta had advanced in development during that time.

The first thing that occurs is a slow leakage from the foetal vessels running in the mesoderm at the surface of the placenta , resulting in the deposit there of coagulated lymph and fine threads of fibrin. At the same time owing to the compression of the placenta from side to side the mesoderm becomes denser in appearance and the vessels are compressed and obliterated. As the interval between the operation and the death of the animal lengthens , this fibrinous invasion of the mesoderm becomes more and more marked , and extends

into all the ramifications of the tissue throughout the placenta , while the cellular elements degenerate. The fibrin deposit in the earlier experiments was always a very fine one, differing entirely from that present in the maternal placenta, but in Experiments VII and VIII , where intervals of 12 and 14 days were allowed to elapse , the deposit in the deeper parts of the foetal placenta differed little from that among the decidual cells in the maternal portion , and the greater part of it was probably derived from the maternal vessels. After such an interval as occurred in those two last experiments the ectoderm has almost entirely been replaced by fibrin tissue.

The changes which the ectoderm undergoes are quite characteristic. They are slower of occurrence than those in the mesoderm. After an interval of 3 days there is only very slight departure from normal. The cells have retained their power of penetrating the maternal tissues , and surrounding the maternal sinuses , and in only very few of them is there any indication of degeneration. From what has been noted regarding the foetus of the punctured sacs , it is evident that all circulation in the foetal vessels of the mesoderm must have ceased shortly after the operation , yet this cessation of circulation for 3 days has resulted in only very slight changes in the ectoderm.

This is what one would expect from the normal method of growth of the ectoderm , for during the first two days that it is penetrating the maternal tissues it is not supplied with a vascular mesodermic covering , and yet during these days its proliferation is extremely rapid.

After a 5 days interval however , as in Experiment II , the signs of degeneration are very apparent , as evidenced by the granular cell protoplasm , and faint or entirely absent nuclear staining. At the same time the power of penetrating the maternal tissues is lost. This loss of power of penetrating the maternal placenta is strikingly shown by a comparison of the relative depths of the foetal and maternal portions of the placentae of the normal and the punctured sacs. In the punctured sacs the two areas are of about equal depth , whereas in the normal sacs the foetal always exceeds the maternal after the 17th day. The degeneration is most marked in the central areas , and least so at the maternal junction where the cells are in close proximity to , and in some cases actually in contact with , the maternal blood.

At intervals of 7 , 9 and 10 days the degenerative changes progressively advance , so that the original structure of the foetal placenta becomes gradually lost , the division into columns is no longer recognisable , and the whole assumes a solid , dense structure , in which no distinct cell outlines

can be made out. After 12 and 14 days the fibrinous deposit everywhere invades the ectoderm , and it is further compressed and strophied , so that it can only be recognised as such here and there near the free surface , the fibrinous deposit always being densest near the maternal junction.

It would thus appear that the ectoderm has very little power of independent growth in the rabbit's placenta during the later stages of gestation , and that it is dependent for its vitality on the foetal vessels contained in the mesoderm which everywhere invades it , unless indeed the changes which the maternal placenta has undergone are responsible for its degeneration.

In the production of hydatid mole and deciduoma malignum in the human subject the death of the foetus is generally regarded as the starting point to be followed by the rapid growth of the epithelial coverings of the villi. Haultain, from the study of a case of deciduoma malignum , concludes that the epithelium of the villi continues to actively proliferate and penetrate the maternal tissues only so long as it is in contact with circulating blood , and that when coagulation and extravasation occur it rapidly degenerates. Chipman, however , believes that in the normal rabbit's placenta it is the fibrinous deposit round the wall of the maternal sinuses that allows of the penetration of the ectoderm - an opinion

which is borne out by a study of the normal growth of the placenta. An excess of fibrinous deposit cannot therefore be regarded as the sole cause of the death and degeneration of the ectoderm in my experiments , a more likely one being the arrest of the circulation in the foetal mesoderm.

There has then been no tendency to proliferation of the foetal ectoderm in these experiments , notwithstanding the fact that the maternal placenta has remained attached to the uterine wall. The inference therefore is that in the human subject conditions other than the mere death of the foetus must be present in order to give rise to hydatid mole and deciduoma malignum, Eden arrives at the same conclusion from the study of a number of retained human placentae , as he was never able to detect any proliferation of the epithelium of the villi in such cases. He concludes that after the death of the foetus the maternal circulation in the intervillous spaces continues actively for some time , but that gradually it becomes slowed in certain areas as a result of the shrinkage of the uterus and the want of stimulus from the growing ovum. The consequence is that the blood coagulates and fibrin is deposited round the villi. He has shown that this is a process which occurs normally in the healthy placenta resulting in the formation of white infarcts. In the placenta of a dead foetus the process goes very much

farther so that the normal spongy consistence is lost and it becomes extensively consolidated. Eden holds there is no attempt at organisation in the fibrin but Fothergill has described the first stages of connective tissue formation. In none of my speciamens has there been any organisation. The fibrinous deposit extends into the compact layer of the decidua , but the spongy layer which is analagous to the zone of separation in the rabbit's placenta is never so invaded but remains loose and easily separable. The villi bathed in maternal blood do not undergo any degenerative change for some time but ultimately they lose their epithelial covering and the connective tissue degenerates , so that the great majority are only recognisable by their outlines; those villi which are situated in a spongy part of the placenta can however scarcely be distinguished from those of a living placenta.

While in these cases of retained human placentae it can only be an inference that the first thing to occur is the death of the foetus , I have been able to prove definitely in these experiments that the changes described in the placenta are certainly secondary to foetal death , as in all cases the dead foetus was only at the stage of development of that of the control one removed at the operation. In all the sacs containing dead foetuses the maternal placenta has gone on growing and developing while the foetal has slowly died

and degenerated , and this is probably the sequence of events in the human placenta under similar circumstances. This point of difference must however always be kept in mind in interpreting the pathology of the human placenta in the light of that of the rabbit , viz. , that in the rabbit the uterus contains other normal placentae and foetuses , so that the placenta of the dead foetus shares in the augmented blood supply which the presence of these others determines.

THE LIQUOR AMNII.

The results of all my experiments have been uniform so far as the liquor amnii is concerned. In five of them , viz. , Experiments I , III , IV , V , and VII in which the amniotic fluid was withdrawn there was no re-secretion , and in two , Experiments II and VIII , in which the yolk sac fluid was aspirated , its secretion was arrested; (in one , viz. , Experiment VI , all the foetal structures had disappeared.)

In all the cases there have been a few drops of fluid in the sac at the time of the death of the animal , this being only such quantity as was left at the time of operation. There has therefore been little absorption from the punctured sacs.

From the fact that the secretion of the fluid was arrested in Experiments II and VIII , where the yolk sac fluid was aspirated , it follows that the determining cause of such arrest in these and in the other cases is not the mere withdrawal of some of the amniotic fluid , and it must be looked for elsewhere.

One possible factor that suggests itself as leading to the arrest is an increased pressure within the sac , resulting from the uterine contraction observed in all the cases. When we remember however , that in a normal sac the fluid is

secreted and has the power of distending and thinning the uterine wall , and that the amount of muscular contraction in the punctured sacs in no case resulted in the thickening of the wall to anything approaching that of the non-pregnant horn , I think it may be inferred that there must be some other cause in addition , and that , were the normal processes at work which lead to the accumulation of the fluid , the amount of muscular contraction present would not be sufficient to counteract them.

What these normal processes are which result in the formation of the liquor amnii is still a matter of dispute , and I shall here indicate some of the more recent work which has been done in the attempt to arrive at a solution of the problem.

The subject has been approached from many different standpoints - from the chemical , the physical , the experimental and the pathological , and notwithstanding , there seems to be almost as much diversity of opinion as ever. There are those who believe in a purely maternal origin , those who hold that it is purely foetal , and a third who take up an intermediate position , and say that it is partly maternal and partly foetal. And further , even those who are agreed as to its foetal origin differ regarding its exact method of production , some stating that it is derived directly from the

foetus , and others that it is through the medium of the foetal placenta. The tendency at the present time is , on the whole , towards regarding the theory of the foetal origin as the correct one , although Williams , in his recent textbook of Obstetrics , says regarding the human liquor amnii " It is now generally admitted that it represents in great part a transudation from the maternal vessels."

The study of the chemistry of the human liquor amnii does not throw much light on the subject , although some have taken the occasional presence of small quantities of urea in it to indicate a renal origin , forgetting that urea may be formed in other transudates and exudates , as for instance in the pleura , where a renal origin can be absolutely excluded.

In animals such as the cow and sheep in which the allantoic fluid can be readily got separate from and unmixed with the amniotic , several observations have been made. Thus Döderlein holds , from his chemical examination of the allantoic fluid of the cow , and of the urine of the foetal calf , that the former is directly derived from the latter , and that urinary secretion goes on during the greater part of the development of the foetus.

Jacque has examined the allantoic and amniotic fluids of the sheep cryoscopically in order to determine their molecular concentration. This is always inferior to that of the

blood either foetal or maternal - allantoic fluid $\Delta = 0.522$; amniotic fluid $\Delta = 0.538$; maternal blood $\Delta = 0.578$; foetal blood $\Delta = 0.623$. From this he concludes that the origin of these fluids is not by transudation but by secretion. Further , by observations conducted at different periods of gestation he has determined that the molecular concentration of the two fluids varies. Thus , during the first stages of development when the allantoic sac communicates freely with the bladder of the foetus , the concentration of the allantoic fluid is higher than that of the foetal urine , and lower than that of the amniotic. At an intermediate stage when the urachus is still patent , and the urethra communicates with the amniotic cavity , the liquor amnii and the liquor allantoidii are of the same concantration , while in the later stages of gestation when the urachus is obliterated but the urethra still communicates with the amniotic cavity , the fluid in the latter is of a lower molecular concentration than the allantoic. From this he concludes that in the early stages the allantoic fluid is derived from the foetal kidneys through the urachus , and that the amniotic is derived from it by abstraction of water , while in the later stages the amniotic fluid is derived directly from the foetal kidneys per bladder and urethram , and that the allantoic is derived from the amniotic by the passage of water back through the

membrane.

These observations I have been able to verify as I took the opportunity of subjecting to cryoscopic examination the amniotic and allantoic fluids from four pregnant sheep at different stages of gestation. The following were my results :-

Length and Weight of Embryo		Amniotic Fluid	Allantoic Fluid
1. 2 cm.	2 grms.	-.388	-.397
2. 7.5 cm.	16 grms.	-.403	-.416
3. 9 cm.	23 grms.	-.379	-.383
4. 20 cm.	--	-.401	-.391

As to whether the conclusions Jacque draws from them are justifiable or not I do not venture to offer an opinion.

While it is thus almost certainly established that the kidneys of the foetal calf and sheep function from an early stage of development , and that probably they supply the allantoic fluid , it is very doubtful whether there is any renal activity in the human foetus. Thus Schaller has demonstrated that by injecting phloridzin into the mother it can be detected in the foetal tissues , and yet no sugar was found in the liquor amnii , which it certainly would have been had the foetal kidneys been secreting urine , and this had passed into the liquor amnii. Many cases too have been recorded where

otherwise healthy children have been born with imperforate urethra or other obstruction in the renal passages , and in these there is often no diminution in the liquor amnii , although in some there is oligohydramnios , as in the case reported by Jaggard , where there was imperforate urethra with absence of one and degeneration of the other kidney.

Bondi concludes from the presence of pepsin and diastase in the liquor amnii , and their absence in the blood of the new born , while they are demonstrable in the serum of the mother , that part at least of the fluid is of maternal origin.

On the other hand Silberstein , from the study of 5½ months uniovular twin pregnancy , in which one of the sacs contained 6½ litres of fluid , while the other contained very little , comes to the conclusion that part at least of the human liquor amnii is derived from the foetal kidneys. In the sac containing the large quantity of fluid the foetus was much larger than the other.- 783 grms. as contrasted with 488. The heart of the polyhydramniotic foetus was 7.5 grms. and that of the oligohydramniotic 3 grms. , the kidneys 2.9 grms. each as against 1 grm. each , and the bladder 2.1 grms. as compared with .2 grms. The glomeruli in the kidneys of the larger foetus had a diameter of 97 to 115 micros , while those of the smaller were only from 65 to 66 micros. He concludes that owing to the arrangement of the vascular areas

of the two foetuses in the common placenta , the larger foetus had an extra supply of blood , part of which was directly transfused from the smaller one , and that as a result all the organs had hypertrophied , probably beginning with the heart. He considers the excess of liquor amnii in the sac as due to the consequent increased activity of the kidneys.

Some recent work on the injection of substances into the mother and the recovery of them in the liquor amnii , on the other hand , seems to support the maternal origin of the fluid. Thus Nicloux injected alcohol into the stomach of pregnant rabbits , and 1½ hours later detected it in the liquor amnii in almost the same proportion as in the serum of the mother. Further , he was able to detect it in the liquor amnii 5 minutes after injection , which would seem to exclude the possibility of its having got there by way of the foetal kidneys.

The experiments of Foerengren by injecting potassium iodide , Bar by injecting potassium ferrocyanide , and Zuntz and Wiener by using sulphate of indigo , have shown that these substances apparently pass into the liquor amnii without passing through the foetus.

A study of the conditions of hydramnios and oligohydramnios in the human subject has not thrown much light on the subject. Silberstein's observation already mentioned , however , seems rather suggestive of the hydramnios being due ,

in some cases at least , to the activity of the renal kidneys. On the other hand , cases of hydramnios have been recorded where there has been imperforate urethra , and so no possibility of the extra fluid being renal in origin. But even though it be proved that in certain more or less pathological conditions the foetus does pass urine into the liquor amnii , this is far from establishing its renal origin in normal cases.

The probable explanation of cases of oligohydramnios is to be found in the passage back of the fluid from the amniotic sac to the mother. Keim concludes from the determination of the freezing points of the maternal blood serum and the liquor amnii at different stages of human gestation that in the early months there is a current from the blood serum to the liquor amnii , and in the later months in the reverse direction. Oligohydrammios may be due to the exaggeration of this latter current , and Billiard , Dieulafe and Gilleo hold that this is due to some disturbance in the foetus , causing it to pass meconium , the presence of which so alters the surface tension of the amniotic fluid as to cause great acceleration of its passage back to the mother. The condition of the foetal kidneys and bladder in cases of oligohydramnios throws no light on its etiology , for while in some cases there has been obstruction in the urethra and hydronephrosis as in those recorded by Ballantyne and Blackwood ,

in others , as in that recorded by Jaggard , like conditions in the foetus have been associated with hydramnios.

It is thus evident that so far no conclusive proof has been forthcoming in favour of either the maternal or the foetal origin , and that the inferences to be drawn from the observations and experiments made are open to dispute. All that my own observations definitely prove is that the secretion of the liquor amnii ceases with the death of the foetus, and this in spite of the fact that the maternal part of the placenta shows very little departure from normal for the first few days after the withdrawal of the fluid. These facts would point towards the conclusion that in the rabbit , at least , the amniotic fluid is foetal in origin , and is not directly derived by a transudation from the maternal vessels , for in the early days after operation there is no pathological condition detected even in the foetal placenta which would prevent such transudation going on did it occur normally. It is however impossible to determine how far the changes which have occurred in the amniotic membrane itself (which is probably not a mere passive structure) have affected the process. The epithelial covering has been lost in those cases in which an interval of 7 days and upwards has elapsed between the death of the foetus , but no other change can be detected.

On the origin of the allantoic fluid the experiments

throw no definite light. In all the cases the fluid has been very small in amount - less then normal - but as it normally diminishes towards the latter part of gestation , no conclusions can be drawn from the fact. It may be that , as Döderlein holds he has proved in the cow , the allantoic fluid in the rabbit is renal in origin , and that the amniotic fluid is derived from it. Certainly there is nothing in the development of the kidneys and ureters to negative this , as at the 11th day when the allantois begins to distend , the glomeruli and tubules are fully formed , and the ureter opens into the genito urinary sinus (Marshall). Subsequent to this the amniotic fluid begins to accumulate.

Such an origin of the two fluids would be quite compatible with the results which I have obtained , but that is all that can be said , and the only certain definite conclusion which I can draw is that the secretion of the liquor amnii in the rabbit is dependent on the vitality of the foetus , that it ceases with its death , and that before any marked changes have occurred in either the maternal or the foetal placenta.

CONCLUSIONS.

1. As a result of the withdrawal of the yolk sac or amniotic fluids the foetus at once dies.

2. Death is due to the arrest of the vitelline and allantoic circulations consequent upon the altered pressure within the sac , and the direct pressure of the uterine wall on the yolk sac wall and placenta.

3. Death of the foetus is rapidly followed by degeneration of all its tissues. The degenerative process is a breaking down of the protoplasm and granular disintegration of the nuclei of the cellular elements; and deposit of the resulting debris , with broken down blood , so as to form a ground substance resembling fibrin. It is in fact , a coagulation necrosis.

4. The organ which disintegrates most rapidly is the liver , and the one which does so most slowly the pancreas , the kidney occupying an intermediate position.

5. Following on its death and degeneration the foetus becomes compressed and flattened out between the uterine

wall and the surface of the placenta.

6. Degenerative changes are much slower of occurrence in the placenta than in the foetus.

7. There is no tendency to the casting off of the placenta immediately after foetal death.

8. The behaviour of the foetal and of the placental portions of the placenta is different.

9. The foetal placenta slowly undergoes a degenerative process , entirely different from anything that occurs in the normal placenta.

10. The first thing that occurs is a deposit of fibrin and debris in the mesodermic tissue , resulting from a leakage from the foetal vessels and the breaking down of cell protoplasm.

11. After this the foetal ectoderm begins to degenerate , the process starting in the middle of the placenta.

12. The ectoderm next the maternal placenta remains normal

in appearance , and has the power of proliferation for 3 days after the death of the foetus.

13. After this the whole of the ectoderm begins to degenerate. The nuclei lose their power of taking up stains , and the protoplasm breaks down.

14. Ultimately the whole of the foetal placenta is invaded by fibrin , a great part of which is derived from the maternal vessels , and the ectoderm is only recognisable in small isolated patches.

15. There is therefore no tendency to proliferation of the ectoderm after the death of the foetus.

16. The changes which the maternal placenta undergoes do not differ in their essential nature from those that occur during its normal development.

17. It continues to grow and develop along the normal lines but the development is accelerated.

18. This acceleration is chiefly evidenced by the more abundant deposit of fibrin , and in the earlier and more

marked differentiation of a zone of separation.

19. The fibrin deposit takes place round the vessels just as in the normal placenta.

20. The placenta becomes detached only when the tissue forming the zone of separation has become sufficiently attenuated , and it does so in the same manner as the normal placenta.

21. Detachment may occur a few days before full time owing to the accelerated development of the placenta.

22. The secretion of the liquor amnii ceases on the death of the foetus.

L I T E R A T U R E

LITERATURE.

Ballantyne , J.W. — Antenatal Pathology and Hygiene. The Foetus. Edinburgh , 1902.

" " " — Antenatal Pathology and Hygiene. The Embryo. Edinburgh , 1904.

" " " — Ed.Med.Jour. XLI , 919

Bar — Recherches pour servir a l'Histoire de l'Hydrammios. Pathogénie , Paris , 1881.

Van Beneden — Recherches sur l'Embryologie des Mammifères. La Formation des Feuillets chez le Lapin. (Arch.de Biologie , 1880).

Van Beneden and Julin — Recherches sur la Formation des Annexes foetales chez les Mammifères. (Lapin et Cheiroptères). (Arch. de Biologie , V).

Blackwood , M. — Ed. Med. Jour. XL , 858.

Bondi — Zent.f.Gyn. , 1903 , 633.

Cathola , M.V. — La Grossesse Extra-membraneuse. (L'Obstétrique , 1902 , 309).

Chipman — Observations on the Placenta of the Rabbit. (Lab. Reports of the Royal College of Physicians Edinburgh , Vol. VIII.)

Dieulafé et Gilleo — Sur la Rôle de la Tension superficielle du Liquide Amniotique dans la Pathogénie de l'Oligohydramnios. (Comp. Rend. de la Soc. de Biolog. , 1905).

Döderlein — Vergleichende Untersuchungen ueber Fruchtwasser und fötalen Stoffswechsel. (Arch. f. Gyn. , 1890 , 141).

Duval — Le Placenta des Rongeurs - Le Placenta du Lapin. (Jour. de L'Anat. et de la Physiologie , 1889-90).

Eden — On the structure of the Ripe Placenta and the Changes which occur in Placentae retained in Utero after the Death of the Foetus. (Lond.Obst.Trans.1896,360).

Foerengren (Comp.Rend.de la Soc.de Biolog. , 1888 543.)

Fothergill Placental Tissue,Fresh and Old. (Brit. Med.Jour.,1896 , I , 1141).

Hart On the Alleged Growth of the Placenta in Extra-uterine Gestation after the Death of the Foetus. (Ed. Obst. Trans. , 1891-92 , 79).

Hart and Gulland The Structure of the Human Placenta. (Trans.Ed.Obst.Soc. , 1891-2).

Haultain Deciduoma Malignum. (Brit.Gyn.Jour. , 1899-1900 , 190).

Hourlier De la Mort du Foetus pendant les derniers Mois de la Grossesse avant le Travail. Thèse,Paris , 1880.

Jacque De la Genèse des Liquides Amniotiques et Allantoidien,cryoscopie et Analyses chémiques. (Bull.de l'Acad.de Biolog. 1902 , 218).

Jaggard (Amer.Jour.Obst. , 1894 , XXIX , 432).

Keim — Cryoscope du Liquide Amniotique. (Bull. de la Soc. d'Obstet. , 1901).

Marchand — Beiträge zur Kenntniss der Placentarbildung. Die Placenta des Kaninchens. Marburg , 1898.

Marshall , Milnes — Vertebrate Embryology , London , 1893.

Masius — De la Genese du Placenta chez le Lapin. (Arch. de Biolog. , 1889).

Martin — De l'Influence des Alterations du Placenta sur la Developpement du Foetus. These , Paris, 1900.

Maximow — Zur Kenntniss des feineren Baues des Kaninchen-Placenta. (Arch. f, mikro. Anat. , 1897).

Maygrier — La Grossess Extra-membraneuse. (L'Obstetrique , 1899 , 303).

Minot — Uterus and Embryo; 1 , Rabbit. (Jour. of Morphology , 1889).

Nicloux — Sur lePassage de l'Alcohol dans la Liquide Amniotique. (Comp. Rend. de la Soc. de Biol. , 1902 , 754).

Perret — La Grossesse Extra-membraneuse. (L'Obstétrique , 1902 , 316.

Robinson , A. — The Nutritive Importance of the Yolk Sac. (Jour. of Anat. and Physiol. 1892 , 308).

Ruge — Ueber den Foetus sanguinolentus. (Zeit. f. Geb. und Gyn. , 1877 , I , 57).

Schaller — Ueber Phloridzin-diabetes , Schwangerer u.s.w. (Arch.f.Gyn. , 1899 , 548).

Schatz — Die Gefassverhandungen der Placentakreislaufe eineiger Zwillinge , ihre Entwickelung und ihre Folgen. (Arch. f. Gyn. , 1887 , 333).

Sentex — Des Causes de la Mort du Produit de la Conception pendant la Grossesse. These, Paris , 1901.

Silberstein (Arch. f. Gyn. , LXVIII , 607).

Webster , J.C. Human Placentation. Chicago , 1901.

Wiener (Arch. f. Gyn. , XVII , 24).

Williams , J. Whitridge Obstetrics , New York , 1904.