CII. ON THE NATURE OF FOETAL RE-ABSORPTION.

By CYRIL NORMAN HUGH LONG AND ALAN STERLING PARKES

From the Department of Physiology, University College, London.

(Received June 12th, 1924.)

INTRODUCTORY.

DURING recent years it has been conclusively shown that following mortality *in utero* a dead foetus may be removed in one or both of two ways. First, and best known, is the process of abortion where a premature parturition entirely clears the uterus. In this case, however, the pregnancy is terminated and in polytocous animals abortion of a dead foetus could only proceed by the sacrifice of the whole litter. In such mammals, therefore, it would not be unexpected to find some other means of removing a dead embryo, and it is certain that in some animals the death of one embryo of a litter is not followed by abortion, in spite of the fact that the embryo disappears. From the fact that atrophic conceptuses in all stages of degeneration may be found in company with normal ones in most polytocous pregnancies, some kind of intra-uterine lysis and re-absorption is inferred.

A provision of this nature would avoid the uneconomic removal of living foetuses in the removal of a dead one higher up the uterus, and it may be said that the *a priori* probability is found to obtain in practice, namely, that in general, abortion occurs in monotocous animals and re-absorption in multiple pregnancies.

There is strong evidence, however, that re-absorption may occur in the early stage of single gestation [Hammond, 1921], and the process has been found to occur in such widely different mammals as mare, cow, sheep, guinea-pig, hamster, rat, mouse, ferret, dog, cat, mole, rabbit and pig [Marshall, 1922].

The number of ova ovulated at one time may be ascertained by counting the corpora lutea¹, and by using this method in conjunction with the actual enumeration of degenerate foetuses found, it has been possible to arrive at some idea of the percentage of foetuses and embryos which have died and been re-absorbed. The following table gives a compilation of the chief investigations on record:

¹ In the ordinary way one corpus luteum represents one ovum ovulated and in polytocous animals the occurrence of two ova in one Graafian follicle and of polyembryony is so rare as to be negligible. The only other source of error lies in the possibility that some ova may not be fertilised, a thing which is probably not common where the sexes are kept together [see Parkes, 1923].

Table I. *Amount of re-absorption of foetuses in various mammals.*

Author	Animal	Foetuses found		Corpora lutea	% death and re-absorption
		Normal	Atrophic		
Hammond [1921]	Sheep	101	8	116	12.9
Hammond [1921]	Pig	267	49	396	32.5
Robinson [1921]	Ferret	1246	—	1643	24.2
Corner [1923]	Pig	3442	43	4480	23.3
Parkes [1923]	Mouse	76	—	82	7.3

With the exception of the last of these investigations the dissections were made at various stages of pregnancy and the figures do not necessarily, therefore, represent the total mortality.

A further method of considering pre-natal mortality consists in comparing the average size of litter at birth with the average corpora lutea count. Some results which have been obtained by this means are given below:

Table II. *Number of corpora lutea and size of litter at birth.*

Author	Animal	Average size of litter at birth	Average corpora lutea	% mortality
Long and Evans [1921]	Rat	6.7	10	33
Hammond [1914]	Pig	12	20	40
Robinson [1921]	Rabbit	5	9.6	37.5
Biedl, et al. [1921]	Rabbit	6.9	9.11	18.33
Robinson [1921]	Ferret	6	9.95	39.23

The second method avoids the inaccuracy involved in dissecting pregnancies in early stages where the mortality which might have occurred later cannot be ascertained, and the higher results obtained are thus explained.

The time incidence of the mortality which does not end in abortion is of considerable interest. Relative to this point Corner [1923] remarks: "...it has become apparent that defective embryos may be found in uteri which are free from infection and other pathological conditions; and that in animals which bear more than one embryo at each gestation normal and abnormal embryos may be found in the uterus at the same time. Furthermore, abnormality or degeneration of the embryo may occur at any stage of gestation." Huber [1915] for the rat and Corner [1921] for the pig, have shown that ova may become abnormal even before implantation. Corner also examined the amount of mortality which had occurred at different stages in pregnancy and found that even in pregnancies where the embryos were not larger than 40 mm. crown-rump length the missing embryos amounted to 20 %. This distribution is confirmed by Robinson's work where 16.03 % of the ova were missing by the eighth day of pregnancy.

Examination of foetuses which are undergoing degeneration and absorption reveals many points which are of importance in considering the actual nature of the process. With this end in view the few facts known regarding such degenerative conceptuses may be considered. It is known:

- (1) That the degeneration is not putrefactive.

(2) That the foetus is the first part of the conceptus to disappear, and the placenta the last.

(3) That the younger the foetus, the more complete is removal.

(4) That the process appears to commence in the liver of the foetus.

(5) That the products of the degeneration are soluble.

The latest stage at which foetuses can be satisfactorily removed is a subject of some controversy, but there is no doubt that moderately young foetuses can be entirely removed. Koebner [1910] goes so far as to say that even the bony structure of later foetuses disappears completely in the rabbit under experimental conditions. Also, there appears to be no limit to the number of conceptuses which can be re-absorbed at once. It has been found experimentally that excision of the corpora lutea causes death of the embryos and complete re-absorption up to the twentieth day of pregnancy in the rabbit [Fraenkel, 1903].

EXTRA-UTERINE AUTOLYSIS OF FOETUSES.

The manner in which the foetal tissues disappear under sterile conditions points at once to the phenomena of autolysis. This indeed was the manner in which Meyer [1919] suggested this process occurred. Now it is known that nearly all the isolated adult organs if incubated at 37° under sterile conditions will in a few days become soluble and disintegrate. The chief organs in which this has been worked out are the muscles, spleen, and liver, especially the last, and the occurrence of this has been attributed to a special class of enzymes—the autolytic enzymes or tissue ferments. These enzymes are probably normal constituents of the organ but only carry out their proteolytic action on the death of the latter.

If foetal absorption is due to the action of such enzymes as these the question arises as to whether the enzymes are foetal or maternal in origin. Are they a constituent of the foetal tissues from the earliest stages, for absorption may occur even of very young embryos, or are they contained in the placenta or uterine wall? From an examination of foetuses in different stages of absorption it is found that absorption begins in the foetus (usually the liver disappears first) and terminates in the placenta which is the last to disappear. Again only dead embryos are absorbed and this also is against the autolytic enzymes being maternal in origin.

The experiments which have been carried out have been to see if autolysis in the laboratory of foetuses removed from the mother follows the same lines as that occurring naturally. The embryos of the rat and mouse, and in one case the pig, have been used. They were incubated at 37° in boiled Ringer's solution to which toluene had been added. As a rule the embryos were left inside the membranes and the placentae were incubated with them. Autolysis rapidly begins and in some 34 hours the liver of the embryo and parts of the intestine have begun to disintegrate. The placentae soon begin to decompose

but generally the membranes remain intact until autolysis has become fairly advanced. In about 7-8 days' time the foetuses have been reduced practically to skeletons and if the medium is renewed and autolysis continued all the tissues disappear except the skeletons.

If the solution in which autolysis is proceeding is examined chemically after about a week's incubation at 37° amino-acids and peptones can be detected.

True enzyme actions are usually greatly inhibited or stopped by the addition of cyanide to the medium in which they are acting.

Foetuses from the same litter were taken (age ten days) and some were incubated at 37° in sterile Ringer-Locke solution only, while others were incubated in Ringer-Locke solution to which about 10 % KCN was added. Autolysis was allowed to proceed for a month and at the end of that time it was found that the foetuses in the Ringer and KCN solution were still intact, even to the membranes and placentae, while those in the normal Ringer were completely disintegrated.

In another experiment a partially absorbed pig embryo (length 12 cm., normal in litter 20 cm.) was taken and autolysis continued in sterile Ringer-Locke for six weeks. At the end of that time the embryo had become much more disintegrated, although complete disintegration had not occurred, probably owing to the products of the autolysis inhibiting further enzyme action.

Further experiments have been carried out in which the livers were removed from pig embryos (75 mm.) and incubated separately, the rest of the tissues being incubated at the same time as the livers. It was found that the liver rapidly autolysed, but while autolysis proceeded as usual in the rest of the embryos it did not go on at the same rate as in the isolated livers.

These, then, are the general data upon which it is possible to base any idea of the actual nature of the process, and the facts all point to the same conclusion, namely, that the dead foetus is rendered re-absorbable by some kind of enzyme activity.

DISCUSSION.

The results of these experiments warrant the conclusion that dead foetuses are removed by autolysis and re-absorption by the maternal organism, especially as conditions in the uterus are ideal for autolysis, viz. sterility and optimum temperature. In the face of the fact that removal is obviously brought about by enzymic activity of some kind there is only one alternative explanation, namely, that the necessary ferments are produced by the uterine wall, an hypothesis which is fraught with very grave objections.

It will be noticed that in two particulars only is there any apparent difference between the observed facts of extra-uterine autolysis and the processes as known *in utero*. First, the placenta goes much earlier in the former case, and second, the disintegration of the foetuses proceeds much further *in utero*. These two discrepancies are easily explained. Normally, the foetal portion of the placenta is in intimate contact with maternal tissues, and may

therefore be supposed to die later than the foetus, whereas, during artificial incubation the placenta, which is known to contain proteolytic enzymes, is the most unstable part of the conceptus. With regard to the second point it is clear that the products of autolysis *in utero* are removed by absorption before any inhibitory concentration is attained. During incubation, on the other hand, the concentration of amino-acids increases to a stage where further breaking down is checked. That this is the explanation of the sub-total disintegration obtained is shown by the experiment where the medium was periodically changed, which resulted in the complete autolysis of the non-skeletal parts of the foetus.

It is clear, however, that the experimental conclusion reached, that the re-absorption is brought about purely by the activity of foetal enzymes, leads to rather dangerous grounds. It is, of course, known that the youngest embryo may be re-absorbed, but is it possible to carry the argument to the logical conclusion and postulate the presence of autolytic enzymes in very young embryos and even in the blastocyst?

The alternative explanation, that autolytic enzymes are present in the uterus ready to remove morbid conceptuses, hardly seems credible, since any such arrangement would form a permanent menace to normal embryos. In the case of older foetuses this problem does not arise, and in these the order of the disappearance makes it clear that some form of autolysis is at work. The mechanism of the removal of the younger embryos is, however, obscure, and upon this problem our experiments throw but little light. The youngest embryos used were those of the pig, about 75 mm. long, younger than anything which can be manipulated for incubation from the mouse. These embryos showed autolysis in the ordinary way, and it seems probable, therefore, that embryos, down at least to a very young age, may be removed by autolysis.

There is little doubt that the capacity for autolysis is a property of foetuses of any species of mammal, but in monotocous animals abortion, since the foetus is converted by death into a foreign body and this is the quickest way of clearing the uterus, usually sets in before autolysis has had time to develop. In polytocous animals, on the other hand, the death of only one or two foetuses does not seem to be sufficient to set up the activity which results in abortion, and as the dead foetus is thus retained autolysis and re-absorption have time to proceed. At the same time the fact that experimental re-absorption of the entire litter in certain polytocous animals may be produced, suggests that these animals have a greater tolerance for the products of autolysis than animals where the death of the product or products of gestation results in abortion.

SUMMARY.

1. It has been shown by various authors that dead foetuses, especially in the younger stages, may be completely removed by some process of intra-uterine absorption.

2. The work recorded in this paper suggests that this process is one of

autolysis on the part of the dead foetus. It was found that dead foetuses will autolyse during sterile incubation, and if the products are removed when the concentration becomes inhibitive, all non-skeletal tissue goes into solution. The probability that foetuses are similarly rendered soluble in the uterus is supported by the fact that conditions in the uterus are ideal for autolysis.

3. The alternative explanation, that dead embryos are rendered soluble by uterine enzymes, is subject to grave objections, and is improbable.

BIBLIOGRAPHY.

- Biedl, Peters, and Hofstatter (1921). *Z. Geburts. Gyn.* 84.
Corner (1921). *Pub. Carnegie Inst. Wash.* No. 276.
— (1923). *Amer. J. Anat.* 31.
Fraenkel (1903). *Arch. Gyn.* 68.
Hammond (1914). *J. Agr. Sci.* 6.
— (1921). *J. Agr. Sci.* 11.
Huber (1915). *J. Morph.* 26.
Koebner (1910). *Arch. Gyn.* 91.
Long and Evans (1921). *Anat. Rec.* 21.
Marshall (1922). *Physiology of Reproduction*, 2nd ed.
Meyer (1919). *Biol. Bull.* 36.
Parkes (1923). *Proc. Roy. Soc. London*, B, 95.
Robinson (1921). *Edin. Med. J.* 26.