III.—On the Source of the Amniotic and Allantoic Fluids in Mammals. By D. Noël Paton, M.D., B. P. Watson, M.D., and James Kerr, B.Sc. (From the Research Laboratory of the Royal College of Physicians of Edinburgh, and the Physiological Department of the University of Glasgow.) *

(Read June 24, 1907. MS. received July 4, 1907. Issued separately December 16, 1907.)

CONTENTS.

	PAGE		PAGE
I. Preliminary	71	(3) The chemical examination of the fluids	84
1. Anatomical considerations	71	(a) Amount and specific gravity	84
2. Pathological evidence	73	(b) Presence and nature of proteins	88
3. Experimental evidence	74	(c) Presence of non-protein nitrogen and its nature	90
(1) Results of withdrawing the fluids	74	(d) Pigments	92
(2) The passage of chemical substances from the maternal blood to the fluids	74	(e) Presence of sugar; its nature, source, and relationship to non-protein nitrogen	92
II. Present Investigations	75	(f) Salts	95
1. The part played by the foetus in the passage of chemical substances from the maternal blood to the fluids	75	III. Summary of Evidence and Conclusions	97
(1) Method	76	Appendix—	
(2) Results of experiments	77	The Method used for Detection and Estimation of Iodide	100
(3) Conclusions	80	Determination of Chloride	100
2. The mode of production of the fluids by the foetus	81	Determination of Total Sulphur	101
(1) Evidence from the molecular concentration of the fluids	82	Determination of Total Phosphorus	101
(2) Results of the injection of chemicals into the maternal blood	83	Determination of Calcium and Magnesium	101
		Determination of Potassium and Sodium	102

I. PRELIMINARY.

1. *Anatomical Considerations.*

In all vertebrates, as the embryo develops at one pole of the egg, it sinks inwards till it becomes completely enclosed in a hood or sac filled with fluid, the amnion. When the sac or true amnion has completely closed over the embryo, it becomes separated from the outer layer, which remains in contact with the covering of the egg as the false or chorionic amnion.

At first the amniotic sac is small and closed, but it rapidly becomes more and more distended with fluid as development advances, and when the hind gut opens upon the

* D. NOËL PATON is responsible for the experiments recorded. The chemical examinations were carried out by him in conjunction with B. P. WATSON, with the assistance of Mr A. PATTERSON. The analyses of the inorganic constituents were made by JAMES KERR, B.Sc., assisted by Mr JAMES C. KIRKCALDIE.

surface of the foetus it communicates directly with the amniotic vesicle. When later the opening of the bowel differentiates from the urinary orifice, both anus and the meatus urinarius are in direct communication with the vesicle.

In reptiles, birds, and mammals another vesicle filled with fluid develops to a greater or less extent. This is the allantois. It is an outgrowth from the posterior end of the gut, which grows out in the mesoblast surrounding the amnion, and it may finally separate the amnion from the periphery of the ovum. From the first it is essentially a continuation of that part of the hind gut which will afterwards form the urinary bladder of the foetus. In the wall of this vesicle, in most mammals, the blood-vessels of the embryo form a connection with the lining membrane of the uterus and produce a placenta.

In most of the lower mammals both vesicles are well developed; but in man and the anthropoid apes the allantois attains a very insignificant size and in man takes no part in the formation of the placenta.

The source of the fluids which fill these vesicles is still doubtful. In reptiles and birds they are necessarily foetal in origin. The occurrence of urea and uric acid in the allantoic fluid of these animals, as was long ago demonstrated by JACOBSON and PREVOST (GUSSEROW, *Arch. f. Gynaekologie*, Bd. iii. p. 244, 1872), shows that in them the allantoic fluid is derived, in part at least, from the kidneys.

In mammals the question is not so simple, since the membranes surrounding one or both of the vesicles are in close relationship to the mucous membrane of the maternal uterus. The possibility is thus suggested that one or both fluids may be derived from the mother—a view which has been upheld by one set of authorities, and which is very commonly accepted in modern text-books of obstetrics. Other observers maintain that the fluids are foetal in origin in the mammal as in the sauropsida.

A study of the anatomical relations of the membranes makes it difficult to accept the former hypothesis without very conclusive evidence in its favour. In most mammals the membrane of the allantois alone is in relation to the maternal mucous membrane; but it is always definitely separate from it, and is easily detachable.

The only connection between the foetal and maternal circulation is through the placenta, which, although it varies greatly in distribution and complexity, is always essentially the same in structure—consisting of tufts of foetal blood-vessels, carried out in the allantoic mesoblast, which have thrust their way into blood-spaces in the mucous membrane of the maternal uterus, so that the foetal blood is brought into intimate relation with the maternal blood.

In all mammals foetal blood-vessels run throughout the wall of the allantois,—both in that part lying against the mucous membrane of the uterus, but separated from it by the chorionic amnion, and also in that part which surrounds the amniotic sac.

In man, while in the very early stage of development the ovum is attached all

round to the maternal mucosa by primitive chorionic villi, at the end of the third month of pregnancy the connection between chorion and mucosa breaks down as the placenta forms, and in all later stages is loose and non-vascular, except at the placental site. The connection between the outer layer of the amnion and the inner layer of the chorion is also loose, and can be readily separated even at full time.

Viewing the question from the anatomical standpoint, it is difficult to see how the fluids in these vesicles can be derived from the maternal blood.

2. *Pathological Evidence.*

This has been chiefly derived from the study of polyhydramnios and oligohydramnios in the human ovum. The most noteworthy of the recent observations on this subject is SILBERSTEIN'S (*Arch. f. Gyn.*, lxxviii. 607, 1903). From a study of a 5½-months uni-ovular twin pregnancy, in which one of the sacs contained 6½ litres of fluid, while the other contained very little, he 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 grms. The heart of the polyhydramniotic foetus was 7.5 grms., and that of the oligohydramniotic one, 3 grms.; the kidneys, 2.9 grms. each, as against 1 gm. 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 diverted 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.

The mere fact of only one of the twin amniotic sacs having an excess of fluid is, of course, strong presumptive evidence of the foetal origin of that fluid; but the evidence afforded that the fluid was secreted by the foetal kidneys is not so convincing. Cases of hydramnios have been recorded in which there has been obstruction in the urethra and hydronephrosis in the foetus (JAGGARD, *Am. Jour. Obst.*, xxix. p. 432, 1894). Like conditions in the foetus have been found associated with oligohydramnios, as in cases recorded by BALLANTYNE (*Ed. Med. Jour.*, xl. 858, 1895) and BLACKWOOD (*Ed. Med. Jour.*, xli. 919, 1896); so that no definite conclusion, either in favour of or against the renal origin of the fluid, can be drawn.

In other cases the hydramnios has been found associated with conditions interfering with the circulation in the umbilical cord, such as excessive torsion, thrombosis of the vein, and cirrhosis of the liver (OPITZ, *Central. f. Gyn.*, 1898, p. 553). It is well known that hydramnios is often associated with malformations in the foetus, such as

spina bifida, anencephaly, etc. But which is the primary condition has never been definitely ascertained. Altogether, very little light has been thrown upon the origin of the fluids from a study of pathological conditions in the human subject.

3. *Experimental Evidence.*

(1) *The Removal of the Fluids.*—It seemed to us that the effects of withdrawing the fluids might throw light upon the question of their origin, and one of us carried out a series of experiments on rabbits (WATSON, *Jour. of Obstet. and Gyn. of the British Empire*, Jan. 1906). The fluids were withdrawn from the uterus by an aspirator needle (the abdomen having been opened) at different periods of pregnancy. In the early stages of pregnancy, the fluid removed was yolk-sac fluid, and in the middle and later stages liquor amnii. In both cases the withdrawal resulted in immediate death of the foetus, and this was followed by a degeneration of the foetal part of the placenta. The maternal part of the placenta, on the other hand, continued to grow and to undergo the various histological changes which occur under normal circumstances. In no case, however, was there any re-formation of the liquor amnii. This would appear to indicate that in the rabbit the fluid must be foetal in origin, since its secretion is arrested by the death of the foetus, in spite of the fact that the foetal part of the placenta does not show marked signs of degeneration for a few days, and the maternal part remains apparently normal up to the time it is cast off.

(2) *The Passage of Chemical Substances from the Mother to the Fluids.*—A large number of experiments have been recorded showing that chemical substances injected into the maternal circulation may appear in the fluids; and from certain of these the conclusion has been drawn that the fluids are formed directly from the maternal blood. The best known of these are by ZUNTZ (*Pflüg. Arch.*, Bd. xvi. p. 548, 1878).

The method he employed was that which had been already used by GUSSEROW in his classical investigations (*Arch. f. Gyn.*, Bd. iii. p. 241, 1872), and according to GUSSEROW by even earlier observers, e.g. MEYER (*Bischoffs Entwicklungsgeschichte*, p. 515). The substance used by ZUNTZ was sodium sulphindigotate, which he injected in large quantities into the veins of pregnant rabbits. He states that the amniotic fluid was coloured blue, while the kidneys and other tissues of the foetus were free of the pigment, and that, when he killed the foetus by injecting into it through the uterine wall caustic potash before injecting the pigment, in one experiment at least the pigment was detected in the fluid.

These observations were confirmed by WIENER for rabbits in the later stage of pregnancy (*Arch. f. Gyn.*, Bd. xvii. p. 24, 1881, and Bd. xxiii. p. 183, 1884). He further showed that the passage of pigment into the foetal fluids was more rapid when the kidneys of the mother were first removed. In two pregnant dogs he entirely failed to find the pigment in the foetal fluids, and he also failed in rabbits during the early stages of pregnancy.

It was thus in rabbits only that the passage of this colouring-matter to the foetal fluids, without its presence having been detected in the foetus, was observed by these investigators.

KRUKENBERG (*Arch. f. Gyn.*, Bd. xxii. p. 39, 1884) criticises their conclusions, pointing out that the recognition of sulphindigotate of soda in tissues is not easy, as was indicated by HEIDENHAIN (*Schultze's Arch.*, Bd. x. p. 35), who stated that, by injecting very small quantities, the urine may be made distinctly blue, while it is impossible to recognise the blue colour in the tissues. KRUKENBERG has repeated this experiment on the rabbit, and finds that, while the urine is distinctly blue, it is impossible to detect the pigment in the kidneys and liver. He does not prosecute the question of whether this is simply due to the small quantity of pigment, or whether "organic substances in close contact with small quantities of the pigment can decolorise it."

He further criticises the *experimentum crucis* of killing the foetus by injecting caustic potash and then finding a slight blue coloration of the amniotic fluid. Certainly it seems possible that a traumatic communication might thus have been established between the maternal vessels and the fluids—a conclusion which is supported by the fact that in all experiments but one the red coloration of the fluid from extravasated blood prevented the detection of the blue pigment.

Opposed to the conclusion of ZUNTZ and WIENER are the experiments of GUSSEROW of injecting benzoic acid into the maternal blood and finding hippuric acid in the foetal fluids. Accepting the conclusion of BUNGE and SMIEDEBERG that the synthesis of this substance takes place in the kidney, this observation favours the view that the fluids are produced from the embryonic kidney.

II. PRESENT INVESTIGATIONS.

The unsatisfactory state of our knowledge as to the source of these fluids induced us to take up the question, and we have studied it by two different methods—first, by a continuation of the method of GUSSEROW, ZUNTZ, and others; and secondly, by a study of the chemistry of the fluids.

1. *The Part played by the Fœtus in the Passage of Substances from the Maternal Circulation to the Fluids.*

The objections to the use of the blue sodium sulphindigotate appeared to us so considerable that we have selected another pigment which is extremely easy to recognise in the smallest quantities, namely, fluorescein. We have also made experiments with three substances which are easily recognised by their colour reaction—sodium iodide, sodium ferrocyanide, and sodium salicylate.

For these experiments we have used the rabbit, guinea-pig, cat, and dog. The two

former are somewhat unsatisfactory, not only on account of their small size, but also from the fact that in both the development of the allantois is very incomplete. The same objection does not apply to the dog or cat, although in our hands the cat proved unsatisfactory, from the small amount of fluids in the later stages of gestation, when alone it was possible to be sure that the animals were pregnant.

The method of procedure was to anæsthetise the animal, to fix a cannula connected with a burette containing the solution to be injected in the proximal end of the jugular vein, and to let the solution run in very slowly. In some experiments one cornu of the uterus, with its contained pregnant sacs, was removed to serve as a check, but in later experiments this was not done.

When a sufficient quantity of the fluid had been run in—a good indication of this being given by the intense staining of the mucous membranes with the fluorescein—the injection was stopped and the animal allowed to live for from 10 to 20 minutes, being kept all the time under the anæsthetic. The uterus was then excised and the animal killed by pushing the anæsthetic.

The uterus was at once removed to a clean table, and each sac was carefully opened and the fluids collected in carefully cleaned vessels, care being taken to prevent any admixture with the maternal blood. The foetuses were then removed, weighed, and if any urine was present in the bladder it was collected. Sometimes the foetal blood was collected.

The presence of fluorescein was recognised by its fluorescent appearance even in the most dilute solution; and the amount was estimated by the intensity of the fluorescence.

Salicylic acid was tested for with a dilute solution of ferric perchloride, and the depth of colour gave a fair index of the amount present.

Sodium ferrocyanide was tested for with ferric chloride.

Sodium iodide was tested for by the method given in the Appendix.

The following experiment may be given *in extenso* as typical:—

EXPERIMENT IX.—Irish terrier bitch, about 13 kilos., supposed to be seven weeks pregnant.

On December 14, anæsthetised at 10.30, and two sacs of the right cornu of the uterus at once excised. A cannula placed in the jugular vein and the following solution run in from a burette:—

Sodium iodide	.	.	.	.	.	.	.	10 grms.
Sodium salicylate	.	.	.	.	.	.	.	5 grms.
Fluorescein	.	.	.	.	.	.	.	0.5 grms.
Water	.	.	.	.	.	.	.	100 c.cm.

The injection began at 11.12, and at 11.42, 150 c.c. had been injected (*i.e.* 15 grms. of sodium iodide, 7.5 grms. sodium salicylate, 0.75 gm. fluorescein). The dog was bled to death at 11.50. Eyes strongly fluorescent; skin and all the organs deeply stained. Blood-serum, bile, and urine intensely fluorescent.

	Fœtus Weight.	Allantoic Amount.	Amniotic Amount.
The two sacs removed at the beginning of the experiment contained :—			
1	109	24	12
2	105	28	15
The six sacs removed after death contained :—			
3	103	23	16
4	102	36	19
5	103	31	15
6	98	31	13
7	91	36	18
8	Fœtus left in sac and fixed in formalin.		

None of the fluids were fluorescent except those of 7, which were distinctly so.

The allantoic and amniotic fluids of 4 and 7 gave a slight but distinct reaction of salicylic acid with ferric perchloride.

The combined urine collected from the bladders was strongly fluorescent, but the urine of 6 was free of fluorescence. The combined urine also gave a strong reaction of salicylic acid with ferric perchloride.

The fœtuses, the allantoic and amniotic fluids, and about 1 c.cm. of the combined urine were sent to Mr KERR, who reports as follows, as regards the presence of NaI :—

Sample.	Presence of NaI.	
	Total.	Per cent.
Fœtuses (1 and 2 check), 142 grms.	none	...
„ (3 to 7), 223 grms.	0·008	0·0036
Fœtal urine, 2 c.c.	0·00008	0·004
Allantoic fluid, 140 c.c.	0·0008	0·00057
Amniotic fluid, 60 c.c.	0·0004	0·0006

EXPERIMENT I.—Guinea-pig, 570 grms., nearly full time. Injected 0·29 gm. sodium ferrocyanide in 4½ minutes. Killed after 15 minutes. Urine and kidney of mother gave marked reaction. Fœtal fluid and fœtal tissues gave no reaction. Maternal placenta gave a green colour.

EXPERIMENT II.—Cat, about 2000 grms., nearly full time. 0·8 gm. sodium ferrocyanide in 13 minutes. Animal killed after 20 minutes. Kidney and urine of mother gave very marked reaction. Fluids, tissues, and urine of fœtuses gave no reaction.

EXPERIMENT III.—Rabbit, about 24 days. Sodium sulphindigotate (not fully saturated), 38 c.c. injected in 11 minutes. The maternal urine was blue.

First sac removed 9 minutes after injection.

Fluids not coloured.

Urine „

Kidney „

Second sac, 19 minutes after injection.

Fluid faint green.

Urine not coloured.

Kidney „

Third sac, 34 minutes after injection.

Fluid not coloured.

Urine „

Kidney „

When mother killed, urine in bladder was green, but the kidneys were not coloured.

EXPERIMENT IV.—Rabbit, about 20 days. Fluorescin, 0·5 per cent., 10 c.c. in 6 minutes. Five minutes after, abdomen opened; abundant fluorescent ascitic exudation found. Ten minutes after, one cornu of uterus with three sacs excised. Membranes appeared fluorescent, but fluid was not so. The foetal blood was not fluorescent. Fifteen minutes after, the other cornu was removed, and gave the same characters. The urine and tissue of the mother were strongly fluorescent.

EXPERIMENT V.—Guinea-pig, about 650 grms. 20 c.c. of 0·5 per cent. fluorescin in 8 minutes. Animal killed after 15 minutes. All the maternal tissues markedly fluorescent. No colour in fluids or in tissues of foetuses.

EXPERIMENT VI.—Rabbit, almost full time. 20 c.c. 10 per cent. sodium iodide in 12 minutes. Animal killed 13 minutes after. Two sacs, one with exomphalotic and anencephalic foetus, other with normal foetus but no fluids.

EXPERIMENT VII.—Rabbit, 12–14 days. One sac excised as control. 20 c.c. sodium iodide (10 per cent.) in 13 minutes. Killed 12 minutes after. Amniotic fluid of six sacs mixed—also allantoic. Report:—

Sample.	Presence of Iodide.
Maternal urine	strongly marked.
Amniotic fluid	none.
Allantoic fluid	distinct trace.
Control foetus	none.
Foetuses (6) after injection	strongly marked.

EXPERIMENT VIII.—Rabbit, 17 days. Control sac excised. 20 c.c. sodium iodide (10 per cent.) in 9 minutes. Animal died 6 minutes after injection stopped. The fluids of the remaining six sacs were mixed. Report:—

Sample.	Presence of Iodide.
Maternal urine	very strongly marked.
Mixed fluids after injection	distinct trace.
Foetus	strongly marked.
Control fluids	none.
Control foetus	none.

EXPERIMENT IX.—Already described in detail.

EXPERIMENT X.—Guinea-pig, nearly full time. One foetus of 92 grms. 20 c.c. of 1 per cent. fluorescin in 10 per cent. sodium iodide in 5 minutes. Animal killed 10

minutes after. Maternal blood fluorescent, but urine not so. Fluids not distinctly fluorescent. Foetal blood distinctly fluorescent. Report:—

Sample.	Presence of Iodide.*
Maternal blood	0·026 gm.
Foetus	none.
Fluids	none.
Foetal blood	0·002 gm.
Maternal urine	0·0003 gm.

EXPERIMENT XI.—Guinea-pig, nearly full time. Two foetuses about 80 grms. 17·5 c.c. of same solution in 5 minutes. Animal killed after 10 minutes. Maternal blood fluorescent. Foetal blood fluorescent. Fluids slightly fluorescent. Report:—

Sample.	Presence of Iodide.*
Maternal blood	0·012 gm.
Foetuses	0·002 gm.
Foetal blood	present.
Fluids	0·00006 gm.

EXPERIMENT XII.—Cat. 45 c.c. solution of sodium iodide 10 per cent., sodium salicylate 5 per cent., sodium sulphindigotate to saturation, were injected in 23 minutes. The animal died. Mucous membranes, all connective tissues, and muscles intensely blue. Medulla of kidney blue, cortex green. Liver and spleen not blue; but when cut and exposed to air the surface of liver became greenish blue. Intestines deep blue, but when left covered by other coils lost their blue colour. Urine intensely blue, with red shimmer. Wall of uterus deep blue. Blood-plasma deep blue.

Four sacs. Foetuses almost full time, weighing about 75 grms. each.

Foetal organs and blood gave no trace of blue.

Foetal urine, about 1 c.c., gave no trace of blue, but one drop gave a faint brown with FeCl_3 , indicating the presence of salicylic acid.

Allantoic fluid of brown colour. No blue tinge. Gave faint deepening of brown with FeCl_3 .

Amniotic fluid.—No trace of blue. Slight brown with FeCl_3 . Report states that there is a considerable quantity of iodide in the maternal urine (17 c.c.) and in the maternal blood (56 c.c.), but only a trace in the allantoic fluid (7 c.c.) and in the amniotic fluids (13 c.c.), and a trace in the four foetuses (300 grms.) = 0·0012 gm.

Twelve experiments have thus been performed—five on rabbits, four on guinea-pigs, two on cats, and one on a dog.

Sodium ferrocyanide has been injected twice, sodium sulphindigotate twice, sodium iodide seven times, fluorescein five times, and sodium salicylate twice.

The following table gives the general result of these experiments, arranged under

* In these two experiments the percentage of iodide present cannot be given, as the weights of the specimens were not noted.

the different substances injected. The intensity of the reaction is indicated by the signs 0, —, +, ++, and +++ indicating the most intense reaction.

Substance Used and Species of Animal.	Fœtus.			Mother.		
	Fluids.	Tissues.	Urine.	Urine.	Kidney.	Blood.
Sodium Iodide.						
Guinea-pig	0	0	—	...	...	+
Rabbit	—	+	...	...	...	+
	Am. 0 All. —	++	...	++	...	...
	—	++	...	+++	...	...
	Am. + All. 0	++	...	...	...	...
	" + " ...	++	...	...	...	...
Dog	" — " —	+	+	+	...	...
Sodium Ferrocyanide.						
Guinea-pig	0	0	...	+	+	...
Cat	0	0	...	+	+	...
Fluorescin.						
Guinea-pig	0	0	...	+	+	+
Rabbit	0	0	...	+	+	+
"	...	...	...	...	...	...
"	...	...	...	...	...	...
Dog	Am. — ? All. — ?	+	+	++	...	++
Sodium Salicylate.						
Rabbit	...	...	...	+	...	...
Rabbit	...	...	...	+	...	...
Dog	Am. — All. —	+	+	++	...	...
Sodium Sulphindigotate.						
Rabbit	?	0	...	+	0	...
Cat	Am. 0 All. 0	0	0	+	+	+

These experiments show (1) that, with the one very doubtful exception of sodium sulphindigotate in one sac in the rabbit in Experiment III., in no case did the substance injected into the maternal circulation appear in the fluids without also being present in the fœtus; and (2) that, when the substance appeared in the fluids, its amount in the foetal tissues or foetal urine was always much greater. The unsatisfactory nature of sodium sulphindigotate for such investigations is well shown by Experiment XII.

They therefore show that the transference of material from the mother to the fluids takes place through the fœtus.

D. J. COPPER (*Der Übergang bestimmtes Stoffe von der Mutter in das Fruchtwasser und in den Fötus*. Utrecht, 1905) gives the results of three quantitative determinations

of iodides in the fluids and in the foetal urine after their injection into two pregnant cows and one pregnant goat. In the two cows he finds the iodide in greater percentage amount in the amniotic fluid than in the urine; while in the goat he finds it in about the same amount in the amniotic, but to a less amount in the allantoic than in the blood of the foetus.

The very consistent results which we obtained with a series of chemical substances, in our opinion, counterbalances the value of these two observations, especially as the method employed by us in the determination of the amount of iodine present is more satisfactory than that used by COPPER.

But COPPER does not attempt to argue in favour of the direct passage of the iodide from the maternal blood, but he believes that it passes through the maternal placenta to the chorionic villi of the foetal placenta, and that from the efferent vessels of this it is carried into the wall of the allantoic vesicle, which surrounds the amnion, and that from there it passes into the fluids.

GUSSEROW (*Arch. f. Gyn.*, Bd. iii. p. 241, 1872) had already indicated the possible importance of the vessels running over the placenta.

COPPER has repeated upon ruminants the experiments previously performed by TOERNNGREN (*C.R. Soc. biol.*, 1888, p. 543) on rabbits, of injecting iodides into the amniotic sac and recovering them in the maternal urine. Such observations seem to prove no more than that iodides are taken up by the blood-vessels in the walls of the allantois and so carried to the placenta to be passed into the maternal circulation. They do not in any way indicate that these fluids are formed from the maternal blood-vessels.*

2. *The Mode of Production of the Fluids by the Foetus.*

It having been shown that the fluids and their constituents are foetal in origin, the question of how they are produced has next to be considered.

(1) They may be produced as a transudation from the blood-vessels coursing in the walls of the vesicles, which, as has been indicated, are purely foetal in nature.

(2) They may be derived from the kidneys of the foetus.

(3) They may be derived from both these sources.

(4) One fluid may be formed from the other to a greater or less extent, or each may derive constituents from the other by diffusion or osmosis through the amniotic and allantoic membranes which separate them.

The question of the parts played by (1) transudation from the blood-vessels of the allantois and (2) by the action of the foetal kidneys may first be considered.

It must be at once admitted that the small amount of fluid which is included in the amniotic sac at the time of its development must have an origin independent of blood-vessels and kidneys, and must be derived from a breaking-down or secretory

* COPPER gives a very excellent summary of the previous work on the subject.

action of the cells of the ovum. With this earliest production of the fluid we are not concerned.

In the sheep, the earliest pregnancies examined by us contained embryos of about 1.5 grm. in weight, and in these the kidneys and the allantoic blood-vessels are both well developed and the urethra differentiated, while there is something less than 10 c.c. of amniotic and about 50 c.c. of allantoic fluid.

Before the end of pregnancy these fluids had increased, the amniotic to an average of about 560 c.c., and the allantoic to 90 or 100 c.c.

In the rabbit, the allantoic circulation and the development of the kidney are completed at about the ninth day.

From an early stage of pregnancy both the factors under consideration are therefore capable of playing a part in the formation of these fluids, and the evidence as to the part played by each may now be examined.

(1) *The Evidence afforded by the Study of the Molecular Concentration of the Amniotic and Allantoic Fluids and of the Maternal and Foetal Blood.*—A good many observations have been carried out along these lines by means of the cryoscope, in the hope of determining whether the fluids are secretions or transudates. The most satisfactory are those by JACQUÉ and those by GRÜNBAUM.

JACQUÉ (*Bull. de l'Acad. de Belgique*, 1902, p. 218) has examined the allantoic and amniotic fluids of the sheep cryoscopically in order to determine their molecular concentration. This he found always inferior to that of the blood, either foetal or maternal—allantoic fluid, Δ 0.522; amniotic fluid, Δ 0.538; maternal blood, Δ 0.578; foetal blood, Δ 0.623. From this he concludes that the origin of the fluids is not by transudation but by renal secretion. Further, by observations carried out at different periods of gestation, he finds 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 lower than that of the foetal urine, and lower than that of the amniotic fluid. At a later period, when the urachus is still patent and the urethra communicates with the amniotic cavity, the liquor amnii and the liquor allantoidis are of the same concentration; while in the later stages of development, when the urachus is closed but the urethra still communicates with the amniotic cavity, the amniotic fluid has a higher 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 fluid is derived from it by the abstraction of water. In the later stages the amniotic fluid is derived directly from the foetal kidneys by the urethra, and the allantoic is derived from the amniotic by a passage of water back through the membranes.

Less satisfactory cryoscopic investigations have been carried out by KEIM (*Bullet. de la Société d'Obstétrique*, 1901), and by BILLARD, DIEULAFÉ, and GILLES (*Comp. Rend. de la Soc. de Biolog.*, 1905), and they conclude that in the early stages of

development there is a current from the maternal blood-serum to the amnion, and in the later stages a current in the reverse direction from amnion to maternal blood-serum.

GRÜNBAUM (*Verhand. de physic. med. Gesellsch. zu Würzburg*, Bd. xxvii., No. 3, p. 161, 1905; abstract *Maly's Jahresbericht* for 1905) found that the amniotic fluid had a lower molecular concentration than the maternal blood. The freezing-point reduction of the blood is 0.53, of the foetal fluids 0.485, and of the foetal urine 0.2.

In the cow, the molecular concentration of the amniotic fluid is nearly that of the blood till the end of pregnancy, when it becomes less. In the allantoic, the molecular concentration is at first very little different from that of the blood, later distinctly less, and towards the end of pregnancy again becoming nearly the same as the blood. So far as they were investigated, the dog, cat, and goat gave the same results as the cow. In the pig, the amniotic fluid shows a lower concentration than the blood in the second half of pregnancy, and the allantoic a lesser concentration. From these observations he concludes that in man the amniotic fluid is a mixture of transudation and foetal urine, and that in the cow the amniotic fluid is entirely a transudation, the allantoic fluid in the beginning a transudation and later chiefly foetal urine.

The author appears to conclude that, when molecular concentration of one of the foetal fluids approaches that of the blood, the fluid is to be regarded as a transudation, while if the molecular concentration is lower an admixture with urine is indicated.

Considering the enormous variations in the relationship between the molecular concentration of the urine and blood in the adult, such a conclusion appears hardly warranted. While normally the Δ of the blood is 0.56° C., and that of the urine 2.3, the Δ of the urine may in the caffeine diuresis or after drinking large quantities of beer fall as low as .160.

Again, the study of the absorption of fluids from serous spaces has shown that the molecular concentration of an effusion may be raised by the absorption from it of water. Thus, alterations in the molecular concentration of the foetal fluids may be capable of explanation in terms of the relative activity of formation on the one hand and absorption on the other.

The conclusions arrived at by the study of the molecular concentration of the fluids have thus a somewhat unsatisfactory basis.

It appeared to us that more light might be thrown upon the question of the relative part played by transudation on the one hand, and secretion by the kidneys on the other, by a more careful analysis of the results of our experiments on the injection of substances into the maternal blood, and a study of the chemistry of the fluids.

(2) *Evidence from the injection of Substances into the Maternal Blood.*—The most convincing experiment is No. IX., on a bitch. Here fluorescein, sodium salicylate, and sodium iodide were injected. The combined urines of the foetuses were very strongly fluorescent, but the fluids of only one pregnancy sac showed a slight fluorescence. The foetal urines gave strongly the reaction for salicylic acid, but the

combined fluids gave only a faint although a distinct reaction. For the sodium iodide the figures show :—

Fœtal urine	.	.	.	.	.	.	.	.	.	·04 per mille.
Allantoic fluid	.	.	.	.	.	.	.	.	.	·0057 „
Amniotic fluid	.	.	.	.	.	.	.	.	.	·0066 „

There was nearly ten times as much in the fœtal urine as in the fluids. Such observations seem to indicate that secretion from the kidneys alone would explain the appearance of these substances in the fluids.

(3) *Chemical Examination of Fluids*.—The marked differences in the chemical constitution of urine and of exudations from blood-vessels justified us in hoping that a study of the chemistry of the fluids would throw important light upon their nature. Although a good many isolated observations on the chemistry of these fluids have been recorded, few systematic studies of the subject have been made. The best of these is by DÖDERLEIN (*Arch. f. Gyn.*, xxxvii., Heft i. p. 141, 1890), who carried out an extended series of observations on the amount, physical characters, and chemical constitution of the fluids in the cow throughout pregnancy. Curiously enough, he failed to observe some important points, and, in our opinion, failed also to appreciate the significance of his own evidence.

KESTIAKOWSKI, in *La Physiologiste Russe*, 1898–99, p. 155, gives a series of analyses of the amniotic fluid of the cow at different stages of pregnancy, and he concludes that in the early part of pregnancy it is derived from the vessels of the placenta, and that in the later months it is an excretion from the organs of digestion. His analyses did not include the investigation of the non-protein nitrogen, and he thus failed to understand the significance of the presence of the urinary constituents in the fluids.

For our investigations we have chiefly used the sheep, because (1) it is easy to get a considerable number of pregnant uteruses from the slaughter-house; (2) in the sheep the allantoic and amniotic sacs are both well developed.

More recently these observations have been extended by the examination of the fluids of the cow, pig, and dog.

The results may be considered under the following heads:—

(a) Amount, character, and specific gravity of each fluid at different stages of pregnancy.

(b) The occurrence or non-occurrence of proteins in the fluids of various animals.

(c) The amount, character, and distribution of the non-protein nitrogen.

(d) The pigments.

(e) The occurrence of sugar; its nature and amount, and its relationship to the non-protein nitrogen.

(f) The ash of the fluids.

(a) *Amount and Specific Gravity of the Fluids*.—The following tables give the results of our observations during 1905–6 on the sheep. Table A gives the individual observation. Table B gives the average result at different stages of pregnancy.

TABLE A.

QUANTITY OF AMNIOTIC AND ALLANTOIC FLUIDS, AND THEIR SPECIFIC GRAVITY.

Amniotic Fluid.			Allantoic Fluid.	
Weight of Fœtus, grms.	Quantity, c.c.	Sp. Gr.	Quantity, c.c.	Sp. Gr.
1.5	2	...	10	...
1.8	30	1008.6	125	1006.4
3.4	30	...	55	1009.4
5	15	...	...	...
8	150	...	80	1012
8	28	1008	20	1010
10	86	1013	132	1012
10	40	...	28	...
12	100	1013	117	1013
13	58	...	45	...
13	60	...	30	...
14	150	1011	59	1014
16	70	1012	16	...
18	130	1011	120	1012
18	135	1012	85	1015
18	140	1011	30	1014
22	50	1012	60	...
25	150	1012	32	...
25	170	1010	16	...
28	305	1011	240	1013
32	70	1010	90	1012
35	180	1012	130	1014
35	210	...	45	...
40	350	1011	60	1014
42	170	1011.5	236	1012.5
47	170	...	14 +	...
50	240	1011	60	1014
53	134	...	56	...
55	260	1011	60	1014
80	300	...	...	...
85	245	...	35	...
153	235	...	...	...
195	250	1012	42	...
196	700	1012	...	...
245	480	...	70	...
250	480	1011	35	1015
253	504	1011	...	...
270	950	1012	20	...
295	400	...	...	...
328	420	1010	...	...
337	340	...	52	...
340	450	...	...	...
390	320	1012	104	1020
390	525	...	54	...
430	475	...	18	...
500	500	1012	60	1016
455	380	...	50	...
478	500	...	70	...

TABLE A.—*continued.*

Amniotic Fluid.			Allantoic Fluid.	
Weight of Fœtus, grms.	Quantity, c.c.	Sp. Gr.	Quantity, c.c.	Sp. Gr.
500	270	1011	90	1021
530	315	1010	...	...
565	650	1011	134	1022
570	220	1012	192	1017
620	350	...	60	...
655	370	...	...	...
720	600	1016	...	...
815	680	...	70	...
865	300	...	106	...
1505	750	1014	60	1024
1670	575	...	54	...
1670	520	...	110	1022
2010	410	1012	145	1020

TABLE B.

AVERAGE AMOUNTS OF AMNIOTIC AND ALLANTOIC FLUIDS, AND THEIR SPECIFIC GRAVITY, AT DIFFERENT STAGES OF PREGNANCY.

Amniotic.				Allantoic.		
Weight of Fœtus, grms.	No. of Observations.	Quantity, c.c.	Sp. Gr.	No. of Observations.	Quantity, c.c.	Sp. Gr.
Below 15	12	57	1011	11	66	1011
15 to 30	8	144	1011	8	73	1013.5
30 to 100	11	205	1011	9	96	1013.4
100 to 300	8	499	1012	4	41	1015
300 to 1000	18	425	1012	13	86	1019
Over 1000	4	564	1013	4	92	1022

DÖDERLEIN (*loc. cit.*, p. 149) found in the cow a marked and rapid increase in the amniotic fluid in the first half of pregnancy, and then a very marked fall towards the end; and an almost continuous increase in the allantoic fluid—an increase which became more marked in the second half of pregnancy, when the amniotic fluid was decreasing.

We have been unable to procure the original papers of MAJEWSKI (*De substantiarum quæ liquoribus amnii et allantoidis insinit*: Dissertatio, Dorpat, 1858) on herbivora, or those of TSCHERNOW (*De liquoribus embryonalium in animalibus carnivoris*: Dissertatio, Dorpat, 1858), referred to by DÖDERLEIN, p. 145.

Our own few observations on the cow support DÖDERLEIN'S results, as is shown in the appended table (C):—

TABLE C.

Weight of Fœtus, grms.	No.	Allantoic.		Amniotic.	
		Present Obser- vations.	DÖDERLEIN.	Present Obser- vations.	DÖDERLEIN.
About 100 . . .	2	332	90	247	160
600 to 700 . . .	3	700	850	2730	2000
3000 to 4000 . . .	3	1800	2000	2500	3200
About 9000 . . .	1	3900	6500	460	1200

In the pig we have made seven observations, which give the following average amounts of fluid in each gestation sac with different lengths of embryo:—

Length of Embryo in cms.	Amniotic Fluid in c.cms.	Allantoic Fluid in c.cms.
2.5	1.1	135
3.0	1.4	130
3.0	1.5	144
8.0	17.0	135
10.0	41.0	170
17.0	75.0	155
24.0	70.0	180

This shows the same increase in the amniotic fluids as occurs in the sheep, with the same comparatively small increase in the amount of allantoic fluid.

Amount.—In the animals examined, the *amniotic fluid* is at first small in amount, but rapidly increases, and in the sheep and cow reaches its maximum about the middle of pregnancy, after which it undergoes no very marked change in the sheep, but very markedly decreases in the cow. The allantoic fluid in the earlier part of pregnancy is larger in amount than the amniotic, but in the later stages it may show hardly any increase, as in the sheep, or it may increase enormously, as in the cow.

Possibly these different relations depend upon the extent to which the amniotic fluid is swallowed by the fœtus and re-excreted through the patent urachus into the allantoic vesicle. It is further possible that, in the middle period of pregnancy, before the muscular structures round the urethra are formed, the urine secreted passes equally into both sacs, but that in the later months in some animals, *e.g.* the cow, it may generally pass into the allantoic sac, and only occasionally by micturition into the amniotic.

Specific Gravity.—The allantoic fluid shows a marked increase in specific gravity. The amniotic fluid shows hardly any increase in its specific gravity.

(b) *Proteins of the Fluids*.—Transudations from blood-vessels contain the proteins of the blood-plasma. In determining the source of the fluids, it was therefore necessary to examine them for the presence or absence of such proteins.

The qualitative examination was made by acidifying and boiling for the detection of albumins and globulins, and by the addition of ESBACH'S solution for the detection of lower proteins.

The following table gives the results :—

TABLE D.

	Allantoic.	Amniotic.
Sheep (61)	+	0
Cow (9)	+	+
Pig (7)	+	+
Cat (2)	+	+
Dog (1)	0	+

KESTIAKOWSKI records the presence of albumin in the amniotic fluid of the cow during the early stages of pregnancy only, and the presence of mucin in the later stages.

The variations in the amount of proteins in the allantoic fluid throughout pregnancy were studied in the sheep by precipitating either by boiling with addition of acetic acid, or by the addition of an equal quantity of a 10 per cent solution of trichloroacetic acid, and weighing.

The following table gives the results :—

TABLE E.

Weight of Fœtus in grms.	Per cent. of Protein in Allantoic Fluid.	Average Percentage.
2	0·125	
10	0·40	
10	0·51	
12	0·45	
13	0·30	
13	0·54	
	—	0·42
40	0·89	
47	0·33	
	—	0·61
200	0·65	
390	0·48	
150 to 650	0·38	
400	0·61	
400 to 800	0·86	
600	0·26	
	—	0·53
1500	0·48	
1670	0·46	
	—	0·47

They show that the amount of protein varies little throughout the greater part of pregnancy.

According to DÖDERLEIN's observations on the cow, the amount of proteins in both fluids rises throughout pregnancy in the amniotic from .042 to .45, and in the allantoic from 0.3 to 1.37.

In the dog, six weeks pregnant with embryos of 35 grms., the allantoic fluid was free of proteins, but the amniotic contained distinct amounts of native proteins amounting to .06 per cent.

In another dog, said to be seven weeks pregnant, and with foetuses averaging 100 grms. in weight, a mere trace of protein was found in the allantoic fluid, while a very distinct amount was present in the amniotic.

In the pig's uterus at various stages of pregnancy both amniotic and allantoic fluids contained native proteins, the former 0.495 per cent., the latter 0.563 per cent.

The proportion of albumin and globulin was determined in four cases in the sheep:—

TABLE F.
PROPORTION OF ALBUMIN AND GLOBULIN IN ALLANTOIC FLUID.

Numbers of Fluids mixed.	Weight of Foetuses, grms.	Total Protein.	Per cent. of Albumin.	Globulin	$\frac{A}{G}$
3	40 to 50	.335	.268	.067	4
5	300 to 500	.610	.460	.150	3
?	500 to 600	.380	.283	.097	3
1	600	.265	.235	.040	5

The fact that coagulable proteins do not exist in the amniotic fluid of the sheep seems to oppose the view that it is formed as a transudation from blood-vessels, because, so far as we at present know, all such transudations contain the proteins of the blood-plasma.

On the other hand, their presence in the allantoic fluid of the sheep, and in both fluids in the other animals observed, does not oppose the view that these fluids are formed in the kidneys.

The opportunity of examining foetal urine is not often afforded, because the bladder is generally empty. DÖDERLEIN (*op. cit.*, p. 155) gives two analyses, one of the urine of a foetus of 15,000 grms. and another of a newly-born calf. In the first there was 0.33 per cent., in the second 0.18 per cent., of proteins. In the urine of two foetal sheep, one of 1505 grms. and one of 1630 grms.—nearly full time—we found coagulable proteins present. This is confirmatory of the old observations of DZARDI in 1806, that alcohol causes a white gelatinous precipitate in the urine of the new-born calf, and of those of VIRCHOW, MARTIN RUGE and BIEDERMANN, and of DOHRN in the new-born child (quoted by DÖDERLEIN, *loc. cit.*, p. 172). JACQUÉ (*loc. cit.*) also records the presence of albumin in the urine of foetal sheep.

(c) *Non-Protein Nitrogen and its Distribution.*—While the absence of native proteins affords evidence of some value against the origin of the fluids by transudation, and while their presence does not militate against the idea that the fluids are of renal origin, a study of the presence of non-protein nitrogen, and the nature of the compounds in which it occurs, should throw light on the relation of the fluids to foetal urine.

The total nitrogen was determined in 5 or 10 c.c. by KJELDAHL'S method. In the allantoic fluids the total proteins were determined by precipitation with trichloroacetic acid, and the nitrogen either estimated directly or calculated from the weight of the precipitate. This was subtracted from the total nitrogen, and the non-protein nitrogen was thus obtained.

The urea nitrogen was determined generally by the method of MÖRNER and SJÖQUIST, sometimes also by the method of BOHLAND. By the former method a considerable quantity of any allantoin present is estimated with the urea, and by the latter method all is probably so estimated. The results of the earlier analyses were rejected, as the amount of fluid taken (5 c.c.) proved to be too small to yield an adequate amount of ammonia for accurate titration.

Generally the urea results by BOHLAND'S method were considerably higher than those by MÖRNER'S, especially in the case of the allantoic fluids.

TABLE G.
DISTRIBUTION OF NITROGEN IN AMNIOTIC AND ALLANTOIC FLUIDS.

Amniotic.				Allantoic.				
Weight of Fœtus.	Total N.	M. and S. N.	Per cent.	Total N.	Protein N.	Non-Protein N.	M. and S. N.	Per cent. of M. and S. to Non-Protein N.
1·8	·0368	·024	64	·0688	...	...	·0324	...
5	·0536	...	...	·137	·093	·044	...	...
8	·048	·0312	65	·106	...	...	·0312	...
8	...	...	...	·126	...	...	·0524	...
10	·0256	·02	78	...	...	...	...	...
10	·0592	·0424	72	·157	·0816	·076	·0536	70
13	...	...	...	·154	·048	·106	·0704	66
13	...	...	...	·171	·086	·085	·0648	76
30	·0256	·02	78	·316	·198	·116	·0592	50
32	·0592	·0508	86	·157	...	...	...	...
35	·0592	...	...	·226	·143	·123	·0536	43
41	·0212	·0156	73	·161	·054	·107	·0744	69
150	·066	·055	83	...	...	...	...	...
195	·048	...	...	·328	·101	·227	...	...
196	·134	...	...	...	...	...	...	...
250	·14	·101	72	·302	·041	·261	·137	52
253	·0648	·062	...	...	...	...	...	...
270	·132	...	...	...	...	...	...	...
328	·0368	·0312	84	...	...	...	...	...
370	·06	·0398	66	·332	·0976	·235	·15	63
390	·076	...	...	·276	·0777	·198	...	...

TABLE G—continued.

Amniotic.				Allantoic.				
Weight of Fœtus.	Total N.	M. and S. N.	Per cent.	Total N.	Protein N.	Non-Protein N.	M. and S. N.	Per cent. of M. and S. to Non-Protein N.
450	...	...	...	·339	·06	·279	...	...
512	·0928	...	...	·434	·137	·297	·16	53
560	·0872	·076	87	·277	·0608	·216	·0704	32
720	·0424	·02	62	...	...	...	...	...
740	·0443	·0356	80	·225	·064	·161	·066	38
1505	...	...	...	·371	·0837	·287	...	60
1670	·1404	·104	74	·227	·073	·154	·0928	...
1670	·102	·066	64	...	...	...	...	...

TABLE H.

PERCENTAGE OF NON-PROTEIN NITROGEN IN AMNIOTIC AND ALLANTOIC FLUIDS AT DIFFERENT STAGES OF PREGNANCY.

Weight of Fœtus, grms.	Amniotic.		Allantoic.	
	No. of Observations.	Per cent. of Nitrogen.	No. of Observations.	Per cent. of Nitrogen.
Below 15	5	·044	4	·077
30 to 100	3	·046	2	·115
100 to 300	6	·097	2	·244
300 to 1000	7	·062	6	·23
Over 1000	2	·121	2	·22

TABLE I.

PERCENTAGE OF M. AND S. NITROGEN IN AMNIOTIC AND ALLANTOIC FLUIDS AT DIFFERENT STAGES OF PREGNANCY, AND ITS RELATION TO THE TOTAL NON-PROTEIN NITROGEN.

Weight of Fœtus, grms.	Amniotic.			Allantoic.		
	No. of Observations.	M. and S. N.	Per cent. of M. and S. N. to Total Non-Protein N.	No. of Observations.	M. and S. N.	Per cent. of M. and S. N. to Total Non-Protein N.
Below 15	4	·029	69	6	·0508	70
30 to 100	2	·033	79	2	·063	55
100 to 300	3	·072	77	1	·137	52
300 to 1000	5	·0405	75	4	·094	46
Over 1000	2	·085	69	1	·092	60

The results of our determination of the non-protein nitrogen show that excretory products are constantly present in both fluids throughout the whole of pregnancy, and that in the sheep, as DÖDERLEIN found in the cow, an increase occurs as pregnancy advances. From the beginning and throughout the whole time the amount is higher in the allantoic than in the amniotic fluid.

The study of the distribution of nitrogen in the various excretory substances is still under investigation, but the observations so far made show—

1st. That in the amniotic fluid somewhere between 70 and 80 per cent. of the nitrogen is in the form of urea (or allantoin?), and that the proportion is not altered throughout the course of pregnancy.

2nd. That in the allantoic fluid only about 50 to 70 per cent. of the nitrogen is in urea and allantoin.

That a large amount of this nitrogen of the amniotic fluid is urea has been demonstrated by the separation of urea as the oxalate.

The constant occurrence of nitrogen in simple combinations, and the fact that a considerable proportion of this is urea, is a strong argument in favour of the view that both allantoic and amniotic fluids are derived from the foetal kidney.

(d) *Pigment.*—At the beginning of pregnancy both allantoic and amniotic fluids are colourless, the amniotic fluid being water-clear and the allantoic slightly turbid; but in the later stages the amniotic fluid of the sheep becomes of an amber-yellow colour. In the cow the allantoic fluid has the same colour, but the amniotic fluid often becomes colourless.

After shaking with ether a brown fluid is left, which gives the spectrum of urobilin. *This further indicates the renal origin of the fluid.*

(e) *Sugar.*—The earliest record of the presence of sugar in the foetal fluids which we have found is by MAJEWSKI (*loc. cit.*, 1858). Subsequent observers, *e.g.* DÖDERLEIN, have failed to observe it.

We have found it to be a constant constituent of both allantoic and amniotic fluids of the sheep, cow, pig, cat, and dog, and of the mixed fluids of the rabbit, ferret, and guinea-pig.

The *variations in its amount* in the two fluids throughout pregnancy have been studied in the sheep. The analyses were made in the usual manner by FEHLING'S method. The results are given in the following tables (K and L):—

TABLE K.
DISTRIBUTION OF SUGAR IN AMNIOTIC AND ALLANTOIC FLUIDS.

Weight of Fœtus, grms.	Amniotic Fluid. Per cent. of sugar.	Allantoic Fluid. Per cent. of sugar.
5	·15	·417
8	·078	·5
10	·058	·29
12	·073	·5
13	·058	·34
35	·08	·37
41	·078	·413
42	·077	·27
80	·083	...
270	·263	...
295	·113	·415
328	·240	...
370	·123	·268
390	·166	·71
450	·21	...
480	·17	...
512	·25	·44
530	·31	...
550	·217	...
560	·265	·634
720	·51	...
740	·161	·247
1505	·347	...
1670	·78	·33

TABLE L.
PERCENTAGE OF SUGAR IN AMNIOTIC AND ALLANTOIC FLUIDS AT DIFFERENT STAGES OF PREGNANCY.

Weight of Fœtus, grms.	No. of Obser- vations.	Amniotic Fluid. Per cent.	No. of Obser- vations.	Allantoic Fluid. Per cent.
Below 15	5	·083	5	·409
30 to 100	4	·079	3	·351
100 to 300	2	·188	1	·415
300 to 1000	11	·235	5	·477
Over 1000	2	·563	1	·33

In the allantoic fluid the amount varies little, being ·409 per cent. in the earlier pregnancies, ·438 per cent. in the later stages.

In the amniotic fluid the percentage is at first small—only ·083—and it rises in the later period to ·287—an increase of more than threefold; but it is always smaller than the percentage in the allantoic, except possibly at the very end of gestation.

In a cow about the middle of pregnancy the percentage was:—

Allantoic	.	.	.	.	.	1·00 per cent.
Amniotic	.	.	.	.	.	0·277 „

Nature of the Sugar.—The sugar in the amniotic and allantoic fluids of the sheep and cow reduces FEHLING's solution, ferments with yeast, and yields crystals with the character of glucosazone. But it is lævo-rotatory and gives SELIWANOFF's lævulose reaction. It is therefore the ketose, lævulose. In the allantoic fluid of the dog, the sugar was in such small amounts that its nature could not be investigated.

GÜRBER and GRÜNBAUM (*Munch. med. Wochen.*, 1904, 377) have already recorded the presence of lævulose in these fluids in the sheep, cow, and goat.

Source of the Sugar.—The following facts indicate that the sugar is derived from the foetus:—

1st. We have found the same sugar in the foetal urine of the sheep to the extent of 0·5 to 0·7 per cent.

2nd. It is present in the foetal blood. By extracting large quantities of foetal blood with alcohol, evaporating with charcoal, and taking up in a small quantity of water, we were able to extract a sugar which was lævo-rotatory and which gave SELIWANOFF's reaction distinctly. The fact that it occurs in larger amounts in the urine than in the blood indicates that its presence is not simply due to the swallowing of amniotic fluid by the foetus. In one case the amount of sugar in the blood was as much as 0·42 per cent.

We have been unable to demonstrate this sugar in the liver. The amount of carbohydrate present in that organ in the foetal lamb seems to be very small. CHIPMAN

TABLE M.

NITROGEN-SUGAR RELATIONS.

Weight of Foetus, grms.	Amniotic.			Allantoic.		
	Non-Protein Nitrogen.	Sugar (G).	$\frac{G}{N}$	Non-Protein Nitrogen.	Sugar (G).	$\frac{G}{N}$
5	·0536	·15	2·7	·044	·417	9
8	·0256	·078	3	...	...	...
10	·0592	·058	0·9	·075	·29	3·8
13	·037	·058	1·5	·085	·34	4
35	·0592	·08	1·3	·123	·37	3
46	·0212	·078	3·6	·107	·413	3·8
270	·132	·263	2	...	...	...
295	·02	·113	5·6	...	...	...
328	·036	·24	6·6	...	...	...
350	·06	·123	2·5	·235	·268	1·1
390	·076	·166	2·1	·198	·71	3·5
512	·0928	·25	2·6	·297	·44	1·4
530	·0536	·31	5·7	...	...	...
550	·02	·217	10·8	...	...	...
560	·0872	·265	3	·2168	·634	2·8
720	·0424	·51	12	...	...	...
740	·0331	·161	4·8	·173	·247	1·4
1670	·1404	·78	5	·154	·33	2·1

(*Reports from the Laboratory of the Royal College of Physicians of Edinburgh*, vol. viii., 1903) found glycogen absent from the foetal rabbit on the 22nd day, but present in the placenta from the 8th day.

The demonstration of the origin of the lævulose from the fœtus and of its presence in such considerable quantities in the foetal urine is further proof that the foetal fluids are derived from the foetal kidneys.

The presence of lævulose as a normal constituent of the blood and urine of the fœtus is of considerable interest in connection with the occurrence of cases of lævulosuria in man without any apparent cause.

The relationship of the sugar to the non-protein nitrogen throughout pregnancy is of interest. While in the amniotic fluid the sugar is increased threefold and the nitrogen undergoes a much smaller increase, in the allantoic fluid the nitrogen is increased more than threefold, while the sugar undergoes only a slight rise.

The proportion between nitrogen and sugar thus varies as is shown in Tables M and N.

TABLE N.

RELATIONSHIP OF SUGAR TO NON-PROTEIN NITROGEN AT DIFFERENT STAGES OF PREGNANCY.

Weight of Fœtus, grms.	G N	
	Amniotic.	Allantoic.
Below 15 . . .	2	5·6
30 to 100 . . .	2·4	3·4
100 to 300 . . .	3·8	...
300 to 1670 . . .	5·7	2·05

(f) *Salts*.—The salts of the foetal fluids in the cow throughout pregnancy have been investigated by DÖDERLEIN (*loc. cit.*, p. 161 *et seq.*). He finds the chlorides are higher in amount in the amniotic than in the allantoic fluid, and that in both fluids the percentage varies little throughout pregnancy. The average, as NaCl, is in the amniotic fluid 0·59 per cent., and in the allantoic 0·23. The sodium behaves like the chlorine. Potassium remains constant at about 0·05 per cent. in the amniotic fluid, but in the allantoic fluid it increases from 0·03 to 0·13. He finds a very marked difference in the percentage of magnesium in the two fluids, it being almost absent from the amniotic, but present in the allantoic to the extent of 0·01 to 0·05 per cent.

Our analyses give the following results. The methods used are given in the Appendix.

TABLE O.
INORGANIC CONSTITUENTS OF AMNIOTIC AND ALLANTOIC FLUIDS.

	Sheep.					Cow.				Pig.
Size of Embryos . . .	1670 grms.	155 to 650 grms.	About 25 grms.	2010 grms.	480 grms.	About 2000 grms.	About 3000 grms.	About 3000 grms.	3200 grms.	
Amniotic Fluid.										
Volume sent, in c.cm. .	400	1000	150	410	333	75	375	378	250	217
Grms. per 100 c.c. of fluid—										
Cl . . .	0·349	0·425	0·432	0·344	...	0·444	0·429	0·429	0·425	0·257
PO ₄ . . .	0·044	0·019	...	0·014	...	...	...	...	0·002	...
SO ₄ . . .	0·094	0·037	...	0·043	0·018	0·012	0·010	0·009	0·032	0·023
Ca . . .	0·005	0·007	0·006	0·007	0·007	0·005	0·006	0·005	0·005	0·011
Mg . . .	...	0·003	0·001	0·003	0·002	0·001	0·002	0·001	0·003	0·002
K . . .	...	0·006	0·083	0·040	...	...	...	...	0·033	...
Na . . .	...	0·295	0·291	0·281	...	...	...	...	0·300	...
Allantoic Fluid.										
Volume sent . . .	...	130	75	145	129	345	318	368	200	450
Cl . . .	...	0·143	0·342	0·088	...	0·317	0·138	0·082	0·082	0·153
PO ₄ . . .	...	0·045	...	0·044	...	...	...	...	0·017	...
SO ₄ . . .	...	0·482	...	0·340	0·285	0·028	0·146	0·117	0·204	0·029
Ca . . .	...	0·010	0·012	0·003	0·035	0·016	0·004	0·013	0·003	0·017
Mg . . .	...	0·018	0·006	0·015	0·022	0·004	0·013	0·016	0·032	0·002
K . . .	...	0·070	0·063	0·152	...	...	...	...	0·006	...
Na . . .	...	0·216	0·222	0·188	...	...	...	...	0·168	...

Our results on the cow and sheep confirm the observations of DÖDERLEIN, as is shown in the accompanying table, in the top line of which the averages of all the figures given by DÖDERLEIN are shown, and in the second line the averages of our results are given—

		Ca.	Mg.	Na.	K.	Cl.	PO ₄ .	SO ₄ .
Amniotic	DÖDERLEIN'S Results .	*·009	*·003	·271	·048	·359	...	...
	Present Results .	·006	·002	·291	·055	·393	·02	·031
Allantoic	DÖDERLEIN'S Results .	*·013	*·032	·125	·083	·140	...	...
	Present Results .	·013	·014	·198	·073	·168	·035	·204

We further find—what DÖDERLEIN did not record—an excess of phosphorus and sulphur compounds in the allantoic fluid. Our single examination of the fluid in the pig does not show the differences in the magnesium and calcium contents of the fluids which are observed in ruminants.

DÖDERLEIN lays great stress upon the distribution of these inorganic constituents, maintaining that the high content of the amniotic fluid in chlorine and sodium indicates its origin as a transudate from the plasma, and that the large amount of magnesium in the allantoic fluid indicates its origin from the kidneys. But VIERORDT

* These results should be lower, as in some cases he gives "traces."

(*Daten und Tabellen*, p. 333) gives a table based upon analyses of SCHMIDT and VOGEL showing a content of NaCl in the blood-plasma of 0·5546 per cent., and in the urine of 1·10 per cent., while in flesh and many other constituents of the body the proportion of magnesium is greater than that of calcium. It seems unsatisfactory to attempt to draw conclusions as to the source of the fluids from their inorganic constituents.

III. SUMMARY OF EVIDENCE AND CONCLUSIONS.

To sum up the evidence at our disposal :—

1. As against the fluids being of maternal origin, it has been shown :—

- 1st. That anatomically this is improbable.
- 2nd. That pathological evidence gives no support to the idea.
- 3rd. That, after the foetus has been killed by withdrawing the fluid, no re-formation of the amniotic fluid occurs, although the maternal uterus and placenta remain normal.
- 4th. That various substances injected into the maternal vessels appear in the fluids only after first being in the foetus.

The only evidence in favour of the maternal origin of the fluids is the experimental work of ZUNTZ and WIENER with sulphindigotate of soda on rabbits—the probable fallacies of which have been explained.

2. As against either of the fluids being formed as a transudation from the foetal blood-vessels in the wall of the allantois may be adduced :—

1st. The absence of the proteins of the blood-plasma from the amniotic fluid of the sheep and from the allantoic fluid of the dog. Their presence in the fluids of other animals cannot be considered as indicating their origin as transudations, since these proteins are present in foetal urine.

2nd. The observations recorded on the molecular concentration of the fluids and on the changes which this undergoes throughout pregnancy afford no evidence of their formation as transudations.

The only evidence of an exchange between these vessels and the fluids is the fact that sodium iodide is so readily absorbed from the fluids and passed through the placenta into the maternal circulation. But the absorption of such a substance into the blood-vessels does not prove that normally fluid passes from these vessels into the foetal fluids, any more than its absorption from the bladder proves that normally the urine is secreted from the bladder wall.

3. That the fluids are of the nature of urine derived by secretion from the foetal kidney is indicated by the following facts :—

- 1st. The anatomical connection of the two cavities with the foetal urinary system.
- 2nd. Both fluids contain a considerable proportion of nitrogen in urea and allied compounds.

3rd. The existence of a peculiar sugar—lævulose—in the fluids, and also in the foetal urine.

4th. Proteins, which are generally, although not always, present in the fluids, have been found in the foetal urine.

5th. GUSSEROW observed that, when benzoic acid is injected into the mother, hippuric acid appears in the foetal fluids.

6th. Such substances as sodium iodide, sodium salicylate, and fluorescein, which, when injected into the maternal circulation, appear in the foetal fluids, are found in much greater abundance in the foetal urine and tissues.

All investigators who have recognised the foetal origin of the fluids admit that the allantoic fluid is at least mainly urinary in nature, but it has been maintained by most that the amniotic fluid is a transudation.

Thus ELLENBERGER (*Vergleichende Physiologie der Haussäugethiere*, Bd. ii. p. 523, 1892) says: "The amniotic fluid is a transudation from the rich cutaneous vessels of the embryo; later it is also possibly derived from the vessels of the allantois which surround the amnion, . . . into which, towards the end of pregnancy, urine and saliva may escape."

DÖDERLEIN also regards the amniotic fluid as a transudation, basing his conclusion entirely upon the correspondence between the salts of the fluid and those of the blood-plasma, and ignoring his own results upon the non-protein nitrogen and the other evidence which is forthcoming.

The last question which must be discussed is a very difficult one. Admitting the origin of both fluids as essentially a secretion from the foetal kidneys, does any exchange go on between the fluids, separated as they are by only a thin double membrane?

The facts to be taken into consideration in dealing with this question seem to be the following. They are diagrammatically represented in fig. 2:—

1st. The relatively rapid increase of the amniotic fluid as compared with the allantoic.

2nd. The steady increase of the sp. gr. of the allantoic fluid without any change in the sp. gr. of the amniotic, and the fact that in the early stages the sp. gr. of the two fluids is the same.

3rd. The steady increase of non-protein nitrogen in the allantoic fluid, and the slighter increase of the nitrogen of the amniotic fluid.

4th. The high proportion of urea nitrogen in the amniotic fluid and the steady decrease in the proportion of urea nitrogen in the allantoic fluid.

5th. The steady increase of sugar in the amniotic fluid, so that at the end of pregnancy it becomes equal to, or greater than, the percentage in the allantoic, which from the beginning remains fairly steady.

6th. The high chlorine content of the amniotic fluid as compared with the allantoic throughout pregnancy, and the low magnesium content of the same fluid.

The higher chlorine and possibly the higher sodium content (DÖDERLEIN, KERR), with

the higher molecular concentration found both by JACQUÉ and GRÜNBAUM (fig. 1), of the amniotic fluid early in pregnancy might cause an osmosis of water from the allantoic to the amniotic sac (fig. 3). This might account for a concentration of the allantoic fluid and the rise in the sp. gr. and in the non-protein nitrogen; and, if the amniotic fluid

FIG. 1.—To show changes in the molecular concentration of the fluids throughout gestation.

were also being added to from the kidneys, it might explain the slight change in the sp. gr. and in the non-protein nitrogen in the amniotic fluid.

On the other hand, diffusion might explain the passage of sugar and of the nitrogen in the form of readily diffusible urea from the allantoic to the amniotic fluid, thus

FIG. 2.

raising the percentage of sugar in the amnion and maintaining the percentage of non-protein nitrogen (fig. 3).

The absence of further increase in the amniotic fluid in the later stages of pregnancy may be due partly to the foetus swallowing the fluid, and, in the cow at least, passing it into the allantoic sac; but it may in part be due to the rise in the molecular concentration of the allantoic fluid (JACQUÉ and GRÜNBAUM) arresting the osmotic stream. This would

FIG. 3.

not interfere with the diffusion of sugar, and hence the percentage of that substance in the amnion would tend to rise till it equalled that in the allantoic fluid. But if such exchanges go on, it is difficult to explain the persistence of the high chlorine content of the amniotic fluid. Why should it not pass into the allantoic fluid? Or is it possible that the passage of water and diffusible solids can take place only from allantois to amnion, but not in the reverse direction?

The whole question of these exchanges is further complicated by :—

- (a) The continuous addition of urine to the allantoic fluid.
- (b) The intermittent addition of urine to the amniotic fluid.
- (c) The possible absorption of allantoic or even of amniotic fluid by the blood-vessels going to the placenta.

These various considerations do not affect our main thesis, that *both fetal fluids are essentially derived from the fœtus through the kidney.*

Our thanks are due to Mr A. M. TROTTER, M.R.C.V.S., for his kindness in procuring for us a considerable amount of the material used in these investigations.

APPENDIX.

THE METHOD USED FOR DETECTION AND ESTIMATION OF IODIDE.

The process employed for the detection and approximate determination of the iodide was as follows :—

The specimen was ignited to ash after addition of solid sodium carbonate. The ash was boiled with water, filtered, concentrated to definite volume.

A measured volume of the solution was taken, such that it contained not more than 0·0005 gramme of iodide calculated as sodium iodide, 1 c.c. of chloroform added, and 1 c.c. of concentrated solution of potassium nitrite, and the mixture acidified with dilute sulphuric acid.

The colour of the resulting solution of iodine in chloroform was then compared with the colour obtained by the same process from a standard solution of iodide.

The smallest quantity of iodide which could be detected by this method was equivalent to 0·000036 gramme of sodium iodide.

A blank experiment was conducted, and gave no indication of iodide.

DETERMINATION OF CHLORIDE.

A measured quantity of fluid was evaporated to dryness in a platinum basin, after addition of some pure solid sodium carbonate.

The residue was carefully carbonised, digested with dilute nitric acid, and to the solution a measured excess of standard silver nitrate solution was added.

The precipitated silver chloride was filtered off, and the excess of silver nitrate in

the filtrate was determined by means of standard ammonium thiocyanate solution, using ferric sulphate solution as indicator.

The difference between the amount of silver nitrate added and the amount found after precipitation of chloride was calculated into the equivalent amount of chlorine.

DETERMINATION OF TOTAL SULPHUR.

The total sulphur was determined by igniting with sodium carbonate and potassium nitrate, dissolving the ash in dilute hydrochloric acid, and precipitating with barium chloride solution.

The weight of barium sulphate obtained, multiplied by the factor 0.41146, gave the proportion of total sulphur expressed as SO_4 .

DETERMINATION OF TOTAL PHOSPHORUS.

The total phosphorus was determined by igniting with sodium carbonate and potassium nitrate, dissolving the ash in dilute nitric acid, and precipitating the boiling-hot solution with a boiling-hot solution of ammonium molybdate in presence of ammonium nitrate and nitric acid.

The precipitated ammonium phospho-molybdate was filtered, washed with a mixture of ammonium nitrate and nitric acid solutions, dissolved in ammonia, the solution heated to the boiling-point, and the ammonium phospho-molybdate re-precipitated by addition of boiling-hot nitric acid solution.

The precipitated ammonium phospho-molybdate was filtered through a Gooch's crucible, washed with ammonium nitrate and nitric acid solution, and heated at 160°C . until the weight was constant.

The weight of ammonium phospho-molybdate found, multiplied by the factor 0.05022, gave the proportion of total phosphorus expressed as PO_4 .

DETERMINATION OF CALCIUM AND MAGNESIUM.

The ash from a portion of the fluid was dissolved in dilute hydrochloric acid, filtered, warmed, made alkaline with ammonia, and then acidified with acetic acid.

The calcium was precipitated by addition of ammonium oxalate, the mixture heated for some time, and then filtered. The precipitate was ignited in a platinum basin, then moistened with dilute sulphuric acid, dried, ignited, and weighed as calcium sulphate.

The weight of calcium sulphate, multiplied by 0.29450, gave the proportion of calcium compounds expressed as Ca.

In the filtrate from the calcium oxalate, the magnesium was precipitated by sodium phosphate and ammonia as magnesium ammonium phosphate.

After standing twenty-four hours this was filtered off, washed with dilute ammonia

solution, ignited in a porcelain crucible over a blowpipe, and weighed as magnesium pyrophosphate.

This, multiplied by the factor 0.21875, gave the proportion of magnesium compounds expressed as Mg.

DETERMINATION OF POTASSIUM AND SODIUM.

A measured volume of the fluid was evaporated to dryness in a platinum basin and carefully ignited over a low flame. The ash was dissolved in dilute hydrochloric acid and filtered. The filter was ignited, dissolved in dilute hydrochloric acid, and the solution filtered.

The *mixed filtrates* were made alkaline with barium hydroxide solution, warmed for some time and filtered. The filtrate was evaporated to small volume, and the excess of barium removed by repeated treatment with ammonium carbonate and filtration. Finally, the filtrate was acidified with hydrochloric acid, made alkaline with ammonia, and evaporated to dryness. The ammonium salts were then burnt off in a platinum basin at a low temperature, and the residue weighed. This residue was dissolved in water and filtered. The filter was washed, burnt, and weighed, and its weight subtracted from that of the undissolved residue.

The resultant is the weight of the mixed alkali chlorides.

The filtrate was treated with excess of hydrochloro-platinic acid, evaporated to dryness on water-bath, treated with 85 per cent. alcohol, and filtered. The precipitate was washed with 85 per cent. alcohol, and dissolved through the paper with boiling distilled water. The solution was collected in a small weighed beaker, in which it was carefully evaporated to dryness, dried at 130° C. and weighed.

The weight of potassium chloro-platinate thus obtained, multiplied by the factor 0.3056, gave the weight of potassium chloride.

By deducting the weight of potassium chloride from the total weight of alkali chlorides obtained above, the proportion of sodium chloride present was determined.

The weight of potassium chloride found, multiplied by the factor 0.52479, gave the proportion of potassium compounds expressed as K.

The weight of sodium chloride found, multiplied by the factor 0.39401, gave the proportion of sodium compounds expressed as Na.