A CONTRIBUTION TO THE STUDY OF THE PROTEIN METABOLISM OF THE FOETUS. THE DISTRIBUTION OF NITROGEN IN THE MATERNAL URINE AND IN THE FOETAL FLUIDS THROUGHOUT PREGNANCY

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I. PRELIMINARY

That the foetal fluids of the allantoic and amniotic sacs are mainly foetal urine may now be considered as definitely proved. Structurally the allantoic sac develops as part of the urinary bladder, while the urethra of the foetus early opens into the amniotic sac. This anatomical evidence is confirmed by the experimental work of Gusserow¹, Doderlein², D. Noël Paton³, and Bruno Wolff⁴.

Since chemical examination of the adult urine throws so much light on the processes of metabolism, it was hoped that the examination of the foetal fluids might help to reveal any characteristic differences between adult and foetal protein metabolism.

II. METHODS

The total nitrogen was determined by Kjeldahl's method; non-protein nitrogen by Kjeldahl's method after precipitating with an equal volume of trichloroacetic acid, the filtrate being tested and found not to give

the biuret reaction; ammonia by Folin's method; urea by the Mörner-Folin method; amino acids (including hippuric acid) and allantoin by an indirect method described by me⁵; amino acids (not including hippuric acid) by the formol titration method, hippuric acid being thus got by difference; creatinine and creatine by Folin's colorimetric method, using a Dubosq colorimeter.

In dealing with the amino acids in the adult urine, the large proportion of hippuric acid which herbivorous urines contain has to be considered. By the method for the estimation of amino acids described by me⁵ and used throughout this work, hippuric acid is estimated along with the other amino acids. It was therefore considered necessary to ascertain what proportion of this amino acid nitrogen was due to the presence of hippuric acid, what to the free monamino acids. To actually isolate hippuric acid is a long and troublesome process, and it is difficult to obtain satisfactory quantitative results. An indirect method was therefore sought for which might prove simpler. This was found in the formol titration method first proposed by Sørensen⁶, and later elaborated by Henriques and Sørensen⁷. This method gives the amino acids not including hippuric acid, as the glycine being linked to benzoic acid does not react with formol. It is clearly possible from the difference in the amino acid nitrogen determined by my method and that determined by titration with formol to ascertain the amount of hippuric acid nitrogen present, and this procedure I have adopted. More recently, Henriques and Sørensen⁸ have modified their method so that by it hippuric acid may be directly determined. The following table gives a comparison of the results got by the two methods:—

Hippuric acid difference method			Hippuric acid formol titration direct		
Mgrs. nitrogen per cent.		Per cent. total nitrogen	Mgrs. nitrogen per cent.		Per cent. total nitrogen
20	=	7	21	=	7.4
36	=	15.7	30	=	13
99	=	34.9	99	=	34.9
15	=	2.1	12	=	1.6

Henriques and Sørensen also show that heating with hydrochloric acid and subsequent titration with formol provides a convenient method for the estimation of the nitrogen present as polypeptides and in the more complex compounds.

III. DISTRIBUTION OF NITROGEN IN THE URINE OF ADULT HERBIVORA

As a preliminary to the study of the distribution of nitrogen in the foetal fluids, an investigation of the urine of the adult was essential. I have been unable to find any complete examination of the distribution of nitrogen in the urine of herbivora, though several workers have investigated the occurrence of specific constituents. Salkowski⁹ made no detailed examination of nitrogen in herbivorous urines, but was concerned chiefly with the presence of oxalic acid in urines which had been kept for a long time, and thence with the occurrence of allantoin and hippuric acid. He found that hippuric acid was present in very considerable quantities. In one case, from a twenty-four hours' urine, he recovered 11.85 grams hippuric acid, containing about 12 per cent. of the total nitrogen present. By evaporation he obtained crystals of allantoin, which previous to this had not been found in cows' urines. Salkowski, however, says it is a normal constituent, the presence of which had probably been overlooked, as was the case with allantoin in the dog's urine.

The urines examined by me were got from sheep, oxen and cows at the slaughter-house. These animals are kept for a few days in the slaughter-house before being killed, so that the food given previous to the collection of the urines was approximately the same in each case. This food consists mostly of hay.

These examinations show that urea averages 83 per cent. of the total nitrogen; that allantoin is almost entirely absent; that the amino acids vary in amount, the variation being apparently mostly in the hippuric acid and probably depends on the amount of benzoic compounds in the food. They average 6.5 per cent. of the total nitrogen, of which more than half is hippuric acid. Creatinine averages 3 per cent. of the total nitrogen, creatine 1.9 per cent. The amount of creatinine is almost constant, but that of creatine varies considerably. The sex of the animals was not determined in the sheep.

These tables for the ox show a very marked difference between the urine of the male and of the female. In the urine of the bullock there is a much smaller proportion of amino acids, only 56 per cent. of which is hippuric acid, while in the cow nearly 90 per cent. is in this form. In the urine of the cow allantoin is present in considerable amounts, while in that of the bullock there is at most only a trace. Creatinine constitutes about 5 per cent. of the total nitrogen and creatine, which

SHEEP

TABLE I.—Sheep's urine
Grs. nitrogen in 100 c.c. urine

No.	Total nitrogen	Ammonia	Urea	Allantoin	Amino acid, including hippuric acid	Amino acid, not including hippuric acid	Hippuric acid	Nitrogen not in these	Creatinine nitrogen	Creatine nitrogen
I	1.708	0	1.412	—	0.140	—	—	0.114	0.038	0.004
II	1.709	0	1.421	0.036	0.058	—	—	0.135	0.044	0.015
III	1.964	0.054	1.686	—	0.164	0.051	0.113	0	0.078	0.075
IV	1.540	0.009	1.272	0	0.085	0.053	0.032	0.086	0.051	0.046

Percentage of total nitrogen

No.	Ammonia	Urea	Allantoin	Amino acid, including hippuric acid	Amino acid, not including hippuric acid	Hippuric acid	Nitrogen not in these	Creatinine nitrogen	Creatine nitrogen
I	0	82.5	—	8.4	—	—	6.7	2.2	0.2
II	0	83.1	2.0	3.6	—	—	7.8	2.6	0.9
III	2.6	84.5	—	8.4	2.5	5.9	0	3.9	3.7
IV	0.5	81.9	0	5.6	3.5	2.1	5.6	3.4	2.9

COW AND OX

TABLE III.—Bovine urine
Grs. nitrogen in 100 c.c. urine

No.	Total nitrogen	Ammonia	Urea	Allantoin	Amino acid, including hippuric acid	Amino acid, not including hippuric acid	Hippuric acid	Nitrogen not in these	Creatinine nitrogen	Creatine nitrogen
Cow { I II III IV	I 1.427	0.025	0.991	0.029	—	0.045	—	0.199	0.087	0.058
	II 0.707	0	0.490	0.037	0.117	0.022	0.095	0	0.053	0.010
	III 2.257	0.009	1.309	0.219	0.192	0.011	0.181	0.393	0.086	0.049
	IV 1.584	0.011	1.094	0.131	0.173	0.024	0.149	0.058	0.044	0.073
Ox { I II III IV	I 1.737	0.024	1.335	0.012	0.106	0.052	0.054	0.149	0.082	0.029
	II 1.491	0	1.213	0.004	0.076	0.025	0.051	0.065	0.053	0.060
	III 0.176	0.006	0.150	0	0.017	0.008	0.009	—	0.006	0.002
	IV 1.818	0	1.546	0.013	0.078	—	—	0.090	0.091	0

Percentage of total nitrogen

No.	Ammonia	Urea	Allantoin	Amino acid, including hippuric acid	Amino acid, not including hippuric acid	Hippuric acid	Nitrogen not in these	Creatinine Nitrogen	Creatine nitrogen
Cow { I II III IV	I 1.7	69.4	2.0	—	3.1	—	14.2	6.0	4.4
	II 0	69.0	5.4	16.9	3.0	13.9	0	7.4	1.5
	III 0.4	58.1	9.6	8.5	0.5	8.0	17.5	3.8	2.1
	IV 1.0	68.7	8.3	11.3	1.2	10.1	3.3	2.8	4.6
Ox { I II III IV	I 1.3	76.8	0.8	5.7	3.0	2.7	8.9	4.7	1.7
	II 0	81.2	0.4	6.4	1.7	4.7	4.6	3.4	4.0
	III 3.5	85.2	0	9.4	4.7	4.7	—	3.5	1.2
	IV 0	85.0	0.7	4.3	—	—	5.0	5.0	0

is generally present, is rather more abundant in the female than in the male.

Salkowski, who found allantoin in the urine of the cows he examined, maintained that it is a normal constituent, but suggested the possibility that it is present in the urine only of milch cows and not in that of bullocks. This occurrence of allantoin in the urine of the female—presumably in calf, as are most cows killed in the slaughter-house—suggested that there may be an absorption from the foetus through the maternal placenta of metabolic products which are excreted by the mother. But two or three observations which I have made as to the occurrence of allantoin in the urines of goats during the period of lactation, and after lactation had stopped, do not support this explanation.

The following are the results:—

				Grs. nitrogen in 100 c.c.			
I. Goat in milk—							
Urea nitrogen	...	...	...	1.654	=	70.0 %	T.N.
Allantoin nitrogen	...	...	...	0.136	=	4.9 %	T.N.
II. Goat in milk—							
Urea nitrogen	...	...	...	3.136	=	79.2 %	T.N.
Allantoin nitrogen	...	...	...	0.152	=	3.9 %	T.N.
III. Goat not in milk and not pregnant—							
Urea nitrogen	...	...	...	0.532	=	70.9 %	T.N.
Allantoin nitrogen	...	...	...	0.063	=	8.4 %	T.N.

These observations show that the amount of allantoin present is very considerable, the proportion of nitrogen in this form being almost the same as that found in the urine of cows.

It must therefore be concluded that the presence of allantoin in the urine of pregnant cows is not due to the excretion of foetal products by the maternal kidney but is a characteristic of the maternal metabolism. The larger amount of hippuric acid in the urine of the cow is probably due to the greater intake of benzoic compounds in hay, &c., during pregnancy.

In considering the relation of the foetal fluids to the adult urine the urine of the female is of the most direct importance.

These tables show that in the cow urea constitutes, on an average, 66 per cent. of the total nitrogen; that allantoin averages 6.3 per cent.; that the amino acids vary somewhat, but on an average amount to 9.8 per cent., and of these hippuric acid constitutes about 87.6 per cent.;

that creatinine averages 5 per cent. of the total nitrogen, and that creatine is generally present in considerable proportions.

The impossibility of fixing an absolute standard of normal urine renders it more difficult to determine the significance of variations in the distribution of nitrogen in the foetal fluids since, although these are primarily produced by the foetal kidney, their character may also be modified by the conditions of maternal metabolism.

IV. THE FOETAL FLUIDS

While both fluids are largely of the nature of foetal urine and the allantoic in its early stages entirely so, it is possible that some of the amniotic fluid may be formed from the membranes before the urethra opens, although the structure of the epithelium does not suggest a secreting function. It is further probable that exchanges take place between the two fluids during pregnancy. This question is considered by Noël Paton³, who indicated the factors which may determine the passage of water from the allantoic to the amnion and the diffusion of sugar and non-protein nitrogen in the same direction. The intermittent addition of foetal urine along with these would account for the increased volume of the amniotic fluid and also the increase in its non-protein nitrogen and sugar content.

The possibility of such exchanges between the two fluids complicates the interpretation of any changes which may occur in their chemical composition. But undoubtedly in its early stages the allantoic fluid is urine alone, and its composition may be safely accepted as giving an indication of purely foetal metabolism.

A. RELATIONSHIP OF THE TOTAL NON-PROTEIN NITROGEN IN THE FOETAL FLUIDS TO THE GROWTH OF THE FOETUS

Mere exchanges between the two fluids will not modify the sum of the metabolic products, and it is of interest therefore to determine whether there is any relationship between the waste nitrogen accumulated in these sacs and the growth of the foetus. Taking Noël Paton's figures for the sheep, as being more complete on this point than those of the present investigation, the following table shows that the waste nitrogen accumulates as the foetus grows, but that the nitrogen per unit of weight markedly decreases. The present observations show the same thing in the cow.

SHEEP			
Weight of foetus	Mean weight	Non-protein nitrogen per 1000 grs.	
Below 15	8	...	9
30—100	50	...	4
100—300	230	...	2
300—1,000	420	...	1.1
Over 1,000	1,700	...	0.5

Cow			
Weight of foetus	Mean weight	Non-protein nitrogen per 1,000 grs.	
100—1,000	400	...	1.7
1,000—2,000	1,731	...	1.0
2,000—4,000	3,475	...	0.9
4,000—6,000	4,800	...	0.7

This may be explained by the more active metabolism of the young and rapidly growing foetus in which all the tissues are protoplasmic and undergoing the active metabolism of growth, whereas later the living tissue of the foetus constitutes a smaller proportion of the body.

This decrease in the nitrogen excreted per unit of weight continues throughout infancy. Camerer shows that from the age of $1\frac{1}{2}$ years this decrease is constant, gradually diminishing till at the age of 15 years the metabolism is the same per unit of weight as in the adult.

Another factor which may influence this decrease is the fact that as pregnancy advances, and the vascular connections between the mother and foetus through the placenta become more fully established, the placenta may more and more play the part of the foetal kidney.

The following table and diagrams show the relationship which exists between the sum of the products accumulated in both sacs and the weight of the foetus:—

TABLE V.—SHEEP

Nitrogen per 1,000 grs. of foetus				
Weight of foetus	Non-protein nitrogen	Urea nitrogen	Allantoin nitrogen	Amino acid nitrogen
235	1.9	0.96	0.4	0.18
660	0.47	0.27	0.07	0.05
860	0.49	0.21	—	0.17
1,100	0.83	0.15	0.15	0.14
1,230	1.3	0.4	0.17	0.5
1,340	0.41	0.18	0.08	—
Full time	0.52	0.07	0.04	0.06

Cow				
Nitrogen per 1,000 grs. of foetus				
Weight of foetus	Non-protein nitrogen	Urea nitrogen	Allantoin nitrogen	Amino acid nitrogen
100	1.54	0.52	—	—
900	1.5	0.5	0.12	—
1,930	1.3	0.52	0.09	0.14
3,200	1.0	0.35	0.16	0.33
3,870	1.1	0.28	0.12	0.22
4,050	0.7	0.13	—	0.22
4,500	0.9	0.13	0.22	0.27
5,600	0.5	0.08	0.11	0.13

It will be seen that the nitrogen of the fluids varies directly with the weight of the foetus till about the middle of pregnancy. Allantoin shows a small but steady rise, but urea after about the middle of pregnancy decreases, its place being taken by the amino acids.

B. VARIATIONS IN THE NITROGENOUS CONSTITUENTS THROUGHOUT PREGNANCY

TABLE VI.—SHEEP
ALLANTOIC FLUID

Mgrs. nitrogen per cent. of fluid									
Weight of foetus	No. examined	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Urea	Allantoin	Amino acid including hippuric	Amino acid not including hippuric	Nitrogen not in these
200-1,000	4	140	123	17.5	43.0	16	32.0	7.0	44.0
1,000-1,350	4	236	209	27.2	30.2	28	66.3	19.5	96.6
Full time	4	299	289	10.5	58.6	36	76.0	40.0	115.0

Percentage of total non-protein nitrogen									
Weight of foetus	No. examined	Protein nitrogen per cent. of total nitrogen	Urea	Allantoin	Amino acid including hippuric	Amino acid not including hippuric	Nitrogen not in these		
200-1,000	4	14.3	33.3	20.3	18.5	7.5	31.4		
1,000-1,350	4	8.8	15.2	12.4	31.7	10.8	42.1		
Full time	4	4.6	21.6	12.3	26.7	14.7	40.2		

AMNIOTIC FLUID									
Mgrs. nitrogen per cent. of fluid									
Weight of foetus	No. examined	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Urea	Allantoin	Amino acid including hippuric	Amino acid not including hippuric	Creatinine nitrogen + creatine nitrogen
60-300	4	33.5	31	2.5	20	2.0	6.0	2.0	0
300-1,000	3	37.0	34	3.0	22	3.6	2.5	7.3	0
1,000-1,350	3	67.0	55	12.0	37	8.3	7.0	7.0	1
Full time	4	94.0	80	14.0	72	2.0	6.0	2.5	—

Percentage of non-protein nitrogen										
Weight of foetus	No. examined	Protein nitrogen per cent. of total nitrogen	Urea	Allantoin	Amino acid including hippuric	Amino acid not including hippuric	Nitrogen not in these	Creatinine nitrogen + creatine nitrogen		
60-300	4	6.7	64.8	7.0	18.3	5.5	18.9	0.2		
300-1,000	3	9.0	63.4	10.3	6.9	7.1	21.6	0		
1,000-1,350	3	14.5	61.7	13.8	9.3	6.6	18.3	1.3		
Full time	4	14.8	88.0	3.6	8.1	2.0	3.0	—		

TABLE VII.—COW
ALLANTOIC FLUID

Weight of foetus	No. examined	Total nitrogen	Mgrs. nitrogen per cent. of fluid						Creatine nitrogen	
			Non-protein nitrogen	Protein nitrogen	Urea	Allantoin	Amino acid including hippuric	Amino acid not including hippuric		Nitrogen not in these
100—1,000	4	78.0	67	11.0	19.6	5.3	14.0	—	14.5	—
1,000—4,000	5	172.6	168	4.2	37.0	17.4	47.4	47.5	66.6	5.5
4,000—6,000	4	219.5	216	3.0	27.7	37.5	58.0	—	93.5	10.0
Percentage of total non-protein nitrogen										
Weight of foetus	No. examined	Protein nitrogen per cent. of total nitrogen	Urea	Amino acid including hippuric		Amino acid not including hippuric		Nitrogen not in these	Creatinine nitrogen	Creatine nitrogen
				Allantoin	Urea	hippuric	hippuric			
100—1,000	4	15.5	38.4	5.2	22.7	—	24.6	—	4.6	—
1,000—4,000	5	2.4	22.7	10.2	26.9	25.3	40.0	—	6.6	2.6
4,000—6,000	4	1.2	12.6	18.0	27.1	—	42.2	—	—	6.0
AMNIOTIC FLUID										
Weight of foetus	No. examined	Total nitrogen	Mgrs. nitrogen per cent. of fluid						Creatine Nitrogen	
			Non-protein nitrogen	Protein nitrogen	Urea	Allantoin	Amino acid including hippuric	Amino acid not including hippuric		Nitrogen not in these
100—1,000	2	20.5	19.5	1.0	12.0	1.0	2.5	—	4.0	0.4
1,000—4,000	5	24.9	23.0	1.9	14.0	1.0	2.4	1	5.8	0.4
4,000—6,000	3	35.6	26.0	9.6	11.6	3.6	6.3	—	4.3	—
Percentage of total non-protein nitrogen										
Weight of foetus	No. examined	Protein nitrogen per cent. of total nitrogen	Urea	Amino acid including hippuric		Amino acid not including hippuric		Nitrogen not in these	Creatinine nitrogen	Creatin nitrogen .
				Allantoin	Urea	hippuric	hippuric			
100—1,000	2	5.0	63.0	5.2	12.3	—	19.5	—	0	1.9
1,000—4,000	5	7.1	59.5	4.7	9.9	3.4	25.9	—	0	2.6
4,000—6,000	3	27.7	42.8	16.3	25.4	—	15.3	—	—	—

Urea nitrogen.—In the amniotic fluid the amount of urea present does not undergo any very marked variation with the advance of pregnancy, and the proportion it bears to the non-protein nitrogen shows a correspondingly small change, a slight decrease taking place towards the end of pregnancy. In the allantoic fluid the amount of urea is very much greater, but the proportion of non-protein nitrogen in this form is very much less than in the amniotic fluid and decreases markedly as the foetus increases in weight. This is true for both the cow and sheep, but the decrease in the proportion of urea nitrogen is much more marked in the allantoic fluid of the cow than in that of the sheep.

Allantoin nitrogen.—The amount of allantoin present in both amniotic and allantoic fluids is very considerable, but, as one would expect, is greater in the allantoic and shows a very marked increase towards the end of pregnancy. In both fluids of the cow the proportion is about the same, and in the later stages of development about three times what it was at the beginning. This increase in the proportion is less well marked in the amniotic fluid of the sheep, and is not found in the allantoic fluid of the sheep.

Amino acid nitrogen (including hippuric acid).—In the allantoic fluids, both of the sheep and cow, the amino acids are abundant, especially towards the middle of pregnancy. The increase in the amount is very rapid in the first half of pregnancy, and becomes slower as the foetus increases in weight. The allantoic fluid of the cow shows little change in the proportion of amino acid nitrogen with the advance of pregnancy, but in the amniotic there is a distinct rise in the later stages. The fluids of the sheep, on the contrary, show a slight increase in the proportion of amino acid nitrogen in the allantoic and in the amniotic very little change.

Hippuric acid nitrogen.—The presence of large amounts of hippuric acid in the adult urine renders it interesting to note the evidences of its presence in the foetal fluids. Gusserow¹ found that the injection of benzoic acid into the maternal circulation caused the appearance in the fluids of hippuric acid, not of benzoic acid, and from this argued that the foetal fluids were formed in the foetal kidneys. In the allantoic fluid of the sheep there is a considerable increase of hippuric acid as pregnancy advances, both absolute and proportionate, but in the amniotic fluid the reverse appears to be the tendency.

Creatine and Creatinine nitrogen.—In the allantoic fluid of the cow, in which most determinations have been made, both creatine and

creatinine are present in very nearly equal amounts, and they apparently increase with the weight of the foetus. In the amniotic fluid creatinine is absent, but creatine is present in small amounts. In the amniotic and allantoic fluids of the sheep no creatinine could be found, but a trace of creatine is present in the later stages of pregnancy.

Ammonia nitrogen.—This was absent in every case examined.

The chief points to be noted in regard to these fluids are—the large amount of amino acids, the presence of allantoin in considerable amount, and the large amount of undetermined nitrogen, i.e., nitrogen not in any of the above substances. In all these points the fluids differ from the adult urine.

Nitrogen not in these substances.—Perhaps the most interesting point is the very high proportion of nitrogen not in any of the ordinary urinary constituents of the adult. In the adult urine, 8·8 per cent. in the cow, 6·0 per cent. in the sheep, of the total nitrogen is in compounds other than those determined. In the early allantoic fluid the proportion in the cow is 24·6 per cent., in the sheep 31·4 per cent., figures which correspond approximately to the difference between the total non-protein nitrogen and that in the phosphotungstic acid filtrate. This large amount of undetermined nitrogen was also observed by Noël Paton, who used either the Morner Sjöqvist method for urea determination or the Bohland methods, which gave quite comparable results. The nature of this I have been able only partially to elucidate. It occurs altogether in the phosphotungstic acid precipitate, and is therefore probably mainly of the nature of polypeptides and diamino acids. Trichloroacetic acid was used to remove the protein, and lower proteins and peptones may therefore be excluded since the filtrate from the trichloroacetic acid gave no biuret reaction.

The possibility that the protein metabolism of the foetus had not gone beyond the stage of chains of amino acids was considered, and the amount of peptide nitrogen was estimated. The method used was that of Henriques and Sørensen⁸.

				SHEEP	
				Polypeptide nitrogen per cent.	Still undetermined per cent.
Allantoic	XIII	...	...	7·9 non-protein nitrogen	35·9 non-protein nitrogen
	XV	...	...	0·6	38·9
	XVII	...	...	13·9	23·6
Amniotic	XIII	...	...	Doubtful	17·7
	XVII	...	...	0	4·6
	XVIII	...	...	7·6	— 2·1
				Cow	
Allantoic	XV	...	...	12·3	15·8
Amniotic	XIV	...	...	6·9	24·2

The peptide nitrogen is quite insufficient to account for more than a small proportion of the undetermined nitrogen. I therefore investigated the nitrogen in the phosphotungstic acid precipitate. The precipitate was decomposed by means of barium carbonate and hydrate, the excess of barium removed with carbon dioxide, and silver nitrate added. The precipitate thus obtained probably consists mainly of the silver salts of the diamino acids.

Cow					
		Silver precipitate nitrogen			Still undetermined
		per cent. non-protein nitrogen			per cent. non-protein nitrogen
Allantoic	X	...	...	17.9	14.3
	VII	...	...	19.0	26.0
Amniotic		...	...	9.4	24.0
				3.5	0.1

Diamino acids are thus seen to be present. The purin nitrogen is quite negligible in amount, and hence the conclusion seems to be that *nitrogenous compounds which are not products of metabolism in the adult are present in the early urine of the foetus*. Their nature will require further investigation.

C. COMPARISON OF THE EARLY ALLANTOIC FLUID WITH THE ADULT URINE

A comparison of the early allantoic fluid with the adult urine is of especial importance, since the former gives undoubted evidence as to the nature of purely foetal metabolism.

Cow								
		Urea	Allantoin	Amino acid, including hippuric	Amino acid, not including hippuric	Creatinine	Creatine	Nitrogen not in these
Early allantoic	...	38.4	5.2	22.7	—	—	—	24.6
				26.9	25.3	4.6	2.6	
Adult urine	...	66.3	6.3	12.7	1.9	5.0	3.2	8.8
SHEEP								
Early allantoic	...	33.3	20.3	18.5	7.5	—	—	31.4
Adult urine	...	83.0	?	6.5	3.0	3.0	1.9	6.7

These results for the allantoic fluids of the cow and sheep are fairly concordant and show:—

1. A small proportion of urea nitrogen in both.
2. A large proportion of allantoin in the sheep, but not in the cow,

in which the increase of allantoin occurs later and coincides with an increase in the allantoin of the amniotic fluid.

3. A high proportion of amino acids other than hippuric acid. The figures for the earliest stage in the cow are unfortunately wanting, but the fact that in the next period the total amino acids were almost the same, and consisted almost entirely of acids other than hippuric—26·9 of amino acids including hippuric corresponding to 25·3 per cent. of amino acids not including hippuric acid—justifies the conclusion that in the early stages, too, a high proportion of amino acids is present.

4. In the cow creatine and creatinine were present in considerable amounts in the second stage of gestation—amounts very similar to those of the adult urine. In the sheep the result is more doubtful.

5. The undetermined nitrogen was high for both sheep and cow, and consisted in part probably of diamino acids and polypeptides.

6. The picture of foetal metabolism thus shown by the chemical composition of the early allantoic fluid is one of low deamidizing power as indicated by the low urea and ammonia output and by the high proportion of amino acids. Since the purines are practically absent, the allantoin may be held to represent the purin metabolism. Its presence in the early urine of the cow in amounts similar to that of the adult probably indicates that the nuclear metabolism is already established in its normal course.

The amniotic fluid throughout resembles the adult urine more closely than does the allantoic. Possibly this is because it is a later foetal urine, and occurs when fuller placental development is masking the purely foetal metabolism by the passage of maternal excretory products.

SUMMARY

1. Certain differences are shown to exist between the distribution of nitrogen in the urine of the bullock and of the cow. The urine of the cow contains a large amount of allantoin and of hippuric acid. In the bullock's urine the amino acid content is much smaller and contains a much smaller proportion of hippuric acid, while allantoin is almost entirely absent.

2. There is an increase in the amount of non-protein nitrogen in the foetal fluids throughout the first half of pregnancy. But the amount of nitrogen per unit of weight of the foetus decreases regularly.

3. The foetal fluids are shown to contain the ordinary urinary constituents of adult urine—urea, allantoin, monamino acids, creatinine, creatine, with, in addition, small amounts of polypeptide nitrogen, nitrogen in diamino acids, and nitrogen in compounds which are not found in the adult urine, and the nature of which has not so far been elucidated.

4. Variations in the distribution of nitrogen in the foetal fluids throughout the course of pregnancy exist, which, in the main, consist of a decrease in the proportion of urea nitrogen with a corresponding increase in the proportion of allantoin and amino acid nitrogen.

5. A study of the early allantoic fluid—the urine of the early foetus—shows, as compared with the adult urine, a low urea content, a high proportion of allantoin and amino acids, and a large amount of undetermined nitrogen.

6. It is therefore concluded that the foetal metabolism differs from that of the adult in the less complete catabolism of the protein and in the greater activity of nuclear metabolism as indicated by the amount of allantoin.

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III—Cow

AMNIOTIC FLUID

Mgrs. nitrogen per cent. of fluid

No.	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Urea	Allantoin	Amino acid including hippuric acid	Amino acid not including hippuric acid	Nitrogen not in these	Creatinine nitrogen	Creatine nitrogen
IX	21	21	0	9	1	4	—	7	0	0.4
XIII	20	18	2	15	1	1	—	1	—	—
XI	15	14	1	10	0	0	—	5	—	—
IV	29	28	1	20	0	3	—	5	—	—
II	23.5	23	0.5	14	2	6	—	1	0	0.4
XIV	34	29	5	19	0	3	—	9	0	—
III	23	21	2	7	3	2	1	9	—	—
XII	41	28	13	16	3	3	—	1	—	—
X	39	32	7	14	1	7	—	10	—	—
VI	27	18	9	5	7	4	—	2	—	—

IV—Cow

AMNIOTIC FLUID

Per cent. of non-protein nitrogen

No.	Weight of foetus	Volume in c.c.	Total nitrogen in total fluid	Non-protein nitrogen, grs. in total fluid	Protein nitrogen per cent. total nitrogen	Urea	Allantoin	Amino acid including hippuric acid	Amino acid not including hippuric acid	Nitrogen not in these	Creatinine nitrogen	Creatine Polypeptide nitrogen
IX	100	530	0.112	0.112	0	42.8	4.8	19.0	—	33.4	0	9.4
XIII	900	1,935	0.401	0.347	10.0	83.3	5.6	5.6	—	5.6	—	—
XI	1,250	3,280	0.505	0.459	6.7	66.6	0	0	—	33.4	—	—
IV	1,930	3,400	1.004	0.966	3.5	71.4	0	10.7	—	17.9	—	—
II	2,013	2,890	0.679	0.665	2.0	60.8	8.7	26.1	—	4.4	0	—
XIV	3,200	2,400	0.816	0.706	14.8	65.5	0	3.4	3.4	31.1	—	6.9
III	3,875	1,900	0.429	0.391	8.7	33.3	14.3	9.5	—	42.9	—	—
XII	4,050	430	0.178	0.120	31.8	57.1	7.1	32.2	—	3.6	—	3.5
X	4,500	620	0.246	0.201	18.0	43.7	3.1	21.9	—	31.3	—	—
VI	5,600	1,590	0.432	0.285	33.4	27.7	38.9	22.2	—	11.2	—	—

VII—SHEEP
AMNIOTIC FLUID
Mgrs. nitrogen per cent. of fluid

No.	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Urea	Allantoin	Amino acid including hippuric acid	Amino acid not including hippuric acid	Nitrogen not in these	Creatinine nitrogen + creatine nitrogen
VI	33	33	0	18	1	4	—	10	0.2
VII	37	30	7	15	3	7	—	5	0
XI	25	25	0	20	2	—	—	3	—
IX	39	36	3	27	—	7	2	0	0
XIV	39	35	4	—	—	—	5	—	—
VIII	39	36	3	20	7	0	0	9	0
XIII	34	—	—	26	2	0	—	6	—
II	36	—	—	21	4	5	—	8	—
•IV	32	30	2	17	4	0	—	9	—
X	97	75	22	54	9	7	5	5	1
•III	74	—	—	42	12	—	—	—	—
V	68	56	12	45	2	8	—	1	—
XVIII	104	92	12	83	—	4	0	5	—
XVII	101	88	13	79	—	5	0	4	—
XV	105	87	18	80	—	—	4	0	—

VIII—SHEEP AMNIOTIC

No.	No. of foetuses	Sum of weight of foetus	Volume in c.c.	Total nitrogen grs. nitrogen in total fluid	Non-protein nitrogen, grs. nitrogen in total fluid	Protein nitrogen per cent. total nitrogen
VI	2	65·5	625	0·21	0·21	0
VII	2	150	500	0·185	0·15	19·0
XI	1	235	415	0·104	0·104	0
IX	1	290	600	0·233	0·216	7·7
XIV	1	300	1,020	0·400	0·357	10·3
VIII	2	375	625	0·315	0·225	7·7
XIII	1	660	320	0·110	—	—
II	1	860	230	0·083	—	—
*IV	2	1,115	730	0·234	0·218	6·3
X	1	1,230	630	0·617	0·476	22·7
*III	1	1,340	330	0·245	—	—
V	1	Full time	480	0·330	0·273	17·7
XVIII	1	Full time	180	0·187	0·166	11·6
		2,050				
XVII	1	Full time	230	0·232	0·202	12·9
		2,500				
XV	2	Full time	500	0·525	0·435	17·2
		6,000				