THE UTERUS OF THE EWE

II.* CHEMICAL ANALYSIS OF UTERINE FLUID COLLECTED BY CANNULATION

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Abstract

The chemical composition of uterine fluid collected from ewes cannulated by three different procedures was studied.

Sodium and chloride were the major inorganic ions present. Smaller amounts of potassium, magnesium, calcium, ammonium, bicarbonate, and phosphate ions were also found. Orcinol-reactive carbohydrate was present in high concentration but only 1–4% could be accounted for as the reducing sugar, glucose. Approximately 5 mM lactate was present in the uterine secretions.

Protein concentration varied from 1 to 3 g/100 ml and some differences existed between uterine fluid and blood serum both in electrophoretic mobility and relative concentration of the various protein components. Uterine fluid also differed in amino acid composition from blood serum and the results suggest that uterine fluid is, at least in part, a true secretion and not merely a blood transudate.

Differences in chemical composition between stages of the oestrous cycle were small and only minor differences were found between fluid collected from the uterine horn and that collected from the body of the uterus. By contrast the composition of fluid collected from ewes with a cannula inserted through the cervical canal differed considerably from that of fluid collected by the other two methods of cannulation and the former method may give a less reliable estimate of the true uterine environment.

I. INTRODUCTION

The quantity of luminal fluid in the uterus is small even in large animals such as the cow (Olds and Van Demark 1957). Thus, early references to the chemistry of uterine fluids in the ewe have been restricted mainly to analyses of uterine washings collected from the anaesthetized animal (Heap 1962; Heap *et al.* 1963). Although this technique can be useful for comparing the relative concentrations of various constituents, the results give no information on the concentration of components in the fluid. Successful collection of uterine fluid from the cannulated uterus has overcome this difficulty and the technique has been used to measure the concentration of some biochemical constituents in the uterine secretion recovered from ewes (Iritani *et al.* 1969).

In Part I of this series (Wales and Restall 1971) three methods of cannulating the uterus of the ewe were described and the patterns of secretion collected from the cannulated uteri were presented. These studies suggested that there may be a local

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effect of the cannula on the uterus. This in turn may influence the composition of the fluid collected and the present paper presents data on the chemical composition of the uterine secretions collected from the ewes used in this former study.

II. Materials and Methods

(*a*) *General*

Fluids were collected during the progress of experiments described in detail in Part I (Wales and Restall 1971). After collection the fluids were stored at −20°C until the chemical analyses were performed. Prior to analyses the fluids were thawed and those showing substantial coloration with blood pigments were discarded. The remaining fluids were pooled as described below.

(i) *Fluid Collected from the Cannulated Uterine Horn*

From the experiment to study the effect of ligation of the uterine horn on the secretion obtained from the cannulated uterine horn, fluids from 12 of the 16 ewes used in the earlier study were analysed; fluid from one ewe in each treatment group was not included. In three of these ewes insufficient secretion occurred, and the fourth ewe was omitted at random from the other group to maintain equal numbers of samples in each treatment group. In each of the remaining 12 ewes, the daily samples collected during the elevated secretion associated with oestrus were pooled to form combined samples for each oestrus. In addition, the secretions obtained during the period between each oestrous flow were pooled to form an interoestrous sample for each ewe. The volume of fluid in the combined samples varied considerably from a maximum of 11·0 ml to a minimum of 0·5 ml for some of the interoestrous samples. With some of these smaller samples, not all chemical analyses could be performed.

Fluid from anoestrous ewes was combined to form samples representing collections over consecutive 3-week periods after the last oestrus in each of the four ewes cannulated. In the three sheep used to compare fluid obtained from the uterine horn with that from the fallopian tube, all collections of each fluid in individual ewes were pooled.

(ii) *Fluid Collected following Cannulation at the Uterocervical Junction*

Fluid from four (ewes 4530, 5714, 1868, and 5662) of the seven ewes cannulated at the utero-cervical junction was subjected to chemical analysis. In each of these four ewes, individual daily collections were pooled to make samples corresponding to the peaks related to the observed periods of oestrus. The fluid collected between the oestrous peaks was also pooled for each of these ewes.

(iii) *Fluid Collected following Cannulation through the Cervical Canal*

Three of the four ewes cannulated through the cervical canal produced sufficient secretion to warrant chemical analyses to be undertaken. In each of these ewes the collections were combined to form two samples, one covering the first half of the collection period, the other the second half. The interval between behavioural oestrus in these ewes varied considerably (see Wales and Restall 1971), and the samples thus formed were not related to the stage of the oestrous cycle.

(*b*) *Analytical Procedures*

Sodium, potassium, calcium, and magnesium were estimated by atomic absorption spectroscopy. Osmotic pressure was determined by freezing point depression using a Knauer osmometer. Chloride and phosphorus were determined colorimetrically (Allen 1940; Schoenfeld and Lewellen 1964). The procedure of Phillips (1958) was used for the preparation of phospholipid extract, and acid-insoluble phosphorus was estimated after precipitation with 20% (w/v) trichloroacetic acid. Bicarbonate was measured by back titration after liberation of all CO_2 with excess acid (Van Slyke 1922). Urea and ammonia were determined by the methods of Grunbaum and Pace (1965) and protein by the biuret method (Wales *et al.* 1961). The amino acid content of the fluids was determined according to the method described by Rosen (1957). Orcinol-reactive carbohydrate was estimated by the method of Hewitt (1937) using two volumes of 20% (w/v) trichloroacetic acid for the separation of acid-soluble and acid-insoluble fractions.

A portion of the fluids was deproteinized with 0·5 vol. of 5% (w/v) $ZnSO_4.7H_2O$ and 0·5 vol. of 0·3N $Ba(OH)_2$ and total reducing sugars (Somogyi 1952), glucose (Huggett and Nixon 1956), and lactic acid (Barker and Britton 1957) were estimated in the neutral filtrates.

Proteins were separated by electrophoresis on cellulose acetate strips (Sepraphore, Gelman Co.) using borate buffer (pH 8·6) and a voltage gradient of 30 V/cm. Electrophoresis was continued for 45 min and the position of the resultant protein bands was detected using Poncheau S stain. After recording the mobilities of the various protein bands, the dye complex was eluted from each band with 0·2N NaOH and the contribution of each protein to the total soluble protein in the sample was estimated by measuring the optical density of the acidified solution at a wavelength of 510 nm in a spectrophotometer.

Amino acids were separated by high-voltage paper electrophoresis of deproteinized samples of the fluids. After electrophoresis for 35 min at a voltage gradient of 100 V/cm on Whatman No. 1 paper using acetic–formic acid buffer at pH 1·85 (Atfield and Morris 1961), the electrophoretograms were dried and developed by dipping in 0·25% ninhydrin in acetone, followed by heating for 20 min at 60°C. The resultant bands were eluted in 75% ethanol containing 12·5 mg/100 ml copper sulphate and the optical density measured at 510 nm in a spectrophotometer. Some amino acids were not separated by this method. In order to obtain the relative proportions of these amino acids, and to check the identity of the amino acids separated by electrophoresis, additional aliquots of the fluids were deproteinized and the amino acids were purified by absorption on ion-exchange resins. After elution from the resin and reduction in volume, the amino acids were separated by high-voltage electrophoresis and the areas corresponding to individual amino acids were eluted with water and dried under air. Aliquots of these fractions were then run on Whatman No. 1 paper in a descending system using n-butanol–acetic acid–water (4 : 1 : 5 v/v), phenol–water (4 : 1 v/v), and ethanol–7·5N ammonia–water (8 : 1 : 1 v/v) as solvents. By the use of the combination of these solvents, it was possible to confirm the identity of the amino acids separated by the high-voltage electrophoresis and to separate most amino acids unresolved by the electrophoretic procedure.

(c) *Statistical Analysis*

In the tables the values for each chemical component are presented as means for the ewes in each treatment group ± standard errors of the means based on the between-animal variance. However, in order to compare the chemistry of fluids collected under different experimental conditions, composite statistical analyses of the data for each chemical component were made using a modified method of unweighted means for disproportionate subclass numbers (Snedecor 1956). The 11 means for each chemical component given in the tables of results were subjected to overall analysis using orthogonal polynomials to isolate the variance associated with individual degrees of freedom. Pooled estimates of the between-animal and within-animal variance were appropriately adjusted and used to test the significance of treatments, the between-animal estimate of error being used when it was significantly greater than the within-animal variance. The levels of significance quoted in the text are based on these analyses.

III. Results

(a) *Inorganic Ions*

The data for the inorganic ions found in uterine secretions are shown in Table 1. In all fluids sodium was the main cation and chloride the main anion. Together these ions made up 80–90% of the electrolytes present in the fluids. Small amounts of potassium, magnesium, calcium, ammonium, bicarbonate, and phosphate ions were present.

The concentration of electrolytes in fluid collected during oestrus from ewes cannulated via the uterine horn was not influenced significantly by ligation of the uterine horn or by the length of the intrauterine portion of the cannula. Fluid collected during the interoestrous period in these ewes was similar in composition to that collected at oestrus except that a small (12%) significant increase ($P < 0·01$) in the

TABLE 1

INORGANIC IONS IN UTERINE SECRETIONS

Mean values ± S.E.M. are given

Technique of cannulation of uterus	Fluid analysed	Osmotic pressure (m-osmole)	Concn. of cations (m-equiv/l)					Concn. of anions (m-equiv/l)		
			Na^+	K^+	Mg^{2+}	Ca^{2+}	NH_4^+	Cl^-	HCO_3^-	PO_4^{3-}
Via horn										
Unligated uterus										
Short cannula	Oestrous flow	363±24	140±6	8·1±1·0	1·4±0·2	2·7±0·7	8·6±6·1	135±12·0	14·0±5·2	4·0±2·5
Long cannula	Oestrous flow	370±26	146±2	10·3±1·4	1·7±0·1	3·7±1·2	3·3±0·6	148±0·3	6·3±1·8	1·9±0·2
Ligated uterus										
Short cannula	Oestrous flow	354±6	153±4	9·4±0·4	1·9±0·1	3·8±0·1	1·9±0·8	146±1·0	8·3±0·8	1·1±0·5
Long cannula	Oestrous flow	359±2	145±1	9·5±0·6	2·2±0·7	3·7±0·1	3·0±0·6	149±5·8	7·0±1·2	1·7±0·2
Via horn	Interoestrous pool	369±33	163±4	7·9±0·5	1·8±0·1	3·0±0·3	4·0±0·8	152±1·3	8·0±1·7	2·7±0·2
	Anoestrous pool	326±11	120±5	10·7±0·5	1·7±0·3	1·4±0·3	7·9±1·6	124±0·8	12·7±4·0	3·6±0·4
At uterocervical junction	Oestrous flow	284±4	139±5	8·7±0·4	2·0±0·2	1·7±0·2	3·6±1·2	117±5·0	7·9±2·0	3·3±1·0
	Interoestrous pool	283±6	101±3	9·0±0·5	1·7±0·7	1·1±0·1	7·2±4·1	115±10·3	11·1±3·3	1·7±0·6
Via cervical canal	Oestrous flow	344±20	121±4	16·2±1·4	1·0±0·1	0·9±0·1	19·1±8·0	113±5·2	17·5±3·7	22·2±1·2
Via horn										
Uterine fluid	Oestrous flow	349±58	141±6	11·4±1·2	2·2±0·6	1·7±0·2	4·1±0·2	118±5·2	4·1±2·2	7·5±1·4
Tubal fluid	Oestrous flow	316±23	140±9	8·9±0·1	1·1±0·1	1·7±0·5	4·1±0·4	127±3·0	7·6±1·3	2·4±0·1

concentration of sodium ions was found. The composition of fluid from anoestrous ewes, however, showed a more marked change. There were significantly lower levels of sodium ($P < 0{\cdot}001$), calcium ($P < 0{\cdot}001$), and chloride ($P < 0{\cdot}001$) in the uterine fluid of anoestrous ewes than in cycling ewes. The ionic content of tubal fluid was similar to that of uterine fluid collected from the same animals except that the phosphate content of tubal fluid was lower than that of uterine fluid ($P < 0{\cdot}01$).

Within the fluids collected following cannulation at the uterocervical junction, the osmotic pressure was lower than that recorded for fluids collected from cannulations via the horn ($P < 0{\cdot}001$) and the levels of sodium and chloride were correspondingly lower. With cannulations via the cervical canal, the levels of sodium and chloride in the uterine secretion were similar to those recorded following cannulation at the uterocervical junction. However, osmotic pressure was higher in these fluids and similar to that collected from the cannulated horn, due mainly to the greater content of potassium, ammonium, bicarbonate, and phosphate.

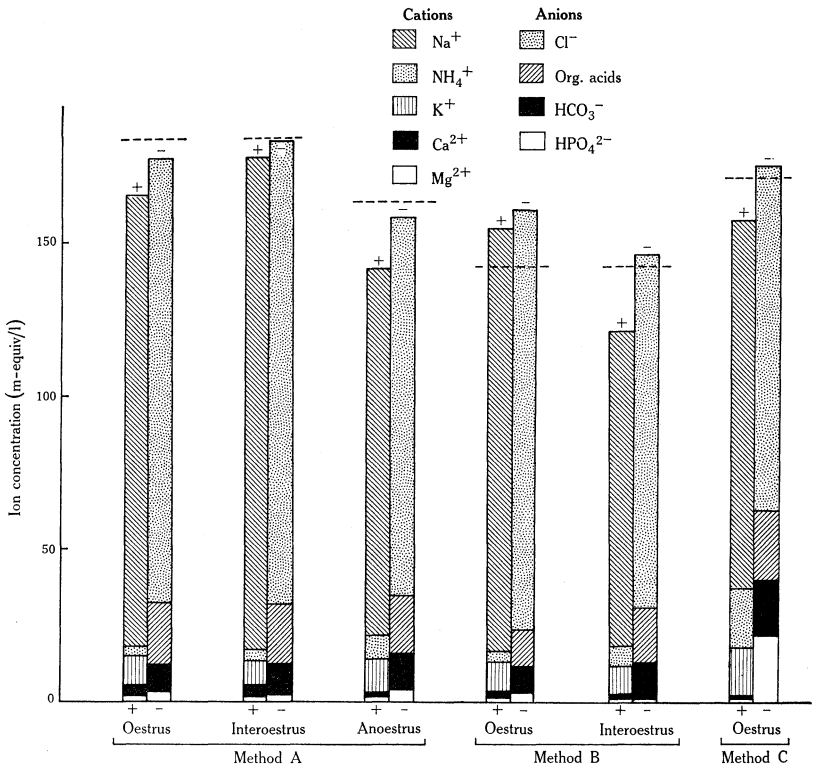

Fig. 1.—Relation between the acid and alkaline equivalents in uterine fluids. The broken horizontal lines indicate the ionic levels equivalent to the measured osmotic pressure. Method A, cannulation of uterus via the uterine horn; method B, cannulation at the uterocervical junction; method C, cannulation via the cervical canal.

The mean acid and alkaline equivalents for each fluid are shown in Figure 1. For the sake of completeness the organic acid equivalents (see Table 5) as well as the

inorganic equivalents have been plotted. In general, there was close acid–base equivalence and the values equate well with those expected from the determination of freezing point depression. It would appear, therefore, that the analyses of cations and anions are reasonably complete.

(*b*) *Distribution of Phosphorus*

The distribution of phosphorus in the uterine secretions is shown in Table 2. The high levels of total phosphorus in fluid collected via the cervical cannula are due to high levels of inorganic phosphorus in this fluid (see Table 1) and this is reflected in the high content of acid-soluble phosphorus. All other fluids contained similar low levels of phosphorus, the average content of total phosphorus in these fluids being approximately 6 mg/100 ml. A major portion of this total phosphorus was acid-soluble, the remainder representing approximately equal portions of non-lipid and lipid acid-insoluble material. In all fractions, the phosphorus content of tubal fluid was significantly lower than that of uterine fluid collected from the same animals.

TABLE 2

DISTRIBUTION OF PHOSPHORUS IN UTERINE SECRETIONS

Mean values ± S.E.M. are given

Technique of cannulation of uterus	Fluid analysed	Phosphorus concn. (mg/100 ml)			
		Total	Acid-soluble	Non-lipid*	Lipid*
Via horn					
Ligated horn					
Short cannula	Oestrous flow	6·9±2·4	7·1±1·7	1·5±0·2	1·7±0·2
Long cannula	Oestrous flow	5·2±0·8	4·7±0·9	1·2±0·4	2·1±0·3
Unligated horn					
Short cannula	Oestrous flow	4·2±1·0	2·7±0·5	1·0±0·1	2·0±0·6
Long cannula	Oestrous flow	4·9±0·8	3·6±0·8	2·6±1·2	2·0±0·3
Via horn	Intercycle pool	7·7±1·6	5·3±0·9	1·3±0·3	3·8±0·8
	Anoestrous pool	6·0±0·8	4·3±0·5	0·6±0·1	1·1±0·4
Dissected cervix	Oestrous flow	4·7±0·1	2·1±0·3	2·4±0·4	1·3±0·2
	Intercycle pool	7·5±2·4	3·4±0·6	1·7±0·8	0·9±0·6
Via cervical canal	Oestrous flow	22·1±2·3	24·3±3·6	1·2±0·2	0·8±0·2
Via horn					
Uterine fluid	Oestrous flow	9·5±1·1	10·4±5·6	1·9±0·8	3·2±0·3
Tubal fluid	Oestrous flow	2·4±0·8	2·3±0·8	0·1±0·1	0·8±0·2

* Acid-soluble fraction.

(*c*) *Organic Compounds*

The results of analyses for a number of organic components in uterine fluids are given in Table 3. In most fluids, there was 100–150 mg/100 ml orcinol-reactive carbohydrate and approximately half of this material was acid-soluble. However, in the case of fluid collected during the interoestrous period after cannulation at the uterocervical junction, much higher levels of acid-soluble carbohydrate were present.

TABLE 3

SOME ORGANIC CONSTITUENTS OF UTERINE SECRETIONS

Mean values ± S.E.M. are given. All values are expressed as milligrams per 100 ml except protein (g/100 ml) and amino acids and urea (mM)

Technique of cannulation of uterus	Fluid analysed	Orcinol-reactive carbohydrate			Total reducing sugar	Glucose	Lactate	Protein	Amino acids	Urea
		Total	Acid-soluble	Acid-insoluble						
Via horn										
Ligated horn										
Short cannula	Oestrous flow	129±14	67±14	71±15	3·9±1·4	2·8±0·7	37±16	2·0±0·4	17·6±4·8	4·8±1·6
Long cannula	Oestrous flow	132±21	82±13	77±9	4·2±1·8	4·9±0·7	49±4	1·8±0·4	16·0±4·2	5·1±0·7
Unligated horn										
Short cannula	Oestrous flow	137±15	74±11	78±10	3·9±0·2	6·2±0·9	40±10	2·2±0·3	15·6±3·1	5·3±0·4
Long cannula	Oestrous flow	133±15	73±5	95±16	3·5±1·2	5·0±0·3	60±5	2·5±0·3	16·6±3·9	4·1±0·6
Via horn	Intercycle pool	162±26	87±12	138±9	5·9±1·0	4·1±0·8	61±4	3·3±0·3	14·5±1·8	3·8±0·6
	Anoestrous pool	110±15	64±11	49±7	6·1±4·8	1·0±0·6	20±11	2·4±0·3	17·0±2·5	2·9±0·5
At uterocervical junction	Oestrous flow	102±4	74±15	41±8	4·6±1·4	0·8±0·6	21±5	1·7±0·5	9·3±1·3	3·0±0·4
	Intercycle pool	327±131	278±85	64±7	9·7±4·0	2·7±1·3	22±8	1·3±0·3	17·0±5·7	6·6±2·5
Via cervical canal	Oestrous flow	117±18	73±12	41±4	9·0±2·1	0·8±0·3	10±5	3·2±0·6	23·1±1·8	4·7±0·4
Via horn										
Uterine fluid	Oestrous flow	174±38	102±6	78±15	3·7±0·5	0·1±0·1	21±2	3·4±0·9	12·1±3·9	1·3±0·6
Tubal fluid	Oestrous flow	70±3	49±5	33±3	3·9±0·4	0·0±0·0	24±2	1·0±0·2	9·4±1·4	3·4±0·2

Only 5–10% of the acid-soluble carbohydrate could be accounted for as reducing sugar. In the secretions collected following cannulation of the uterine horn, glucose was the main reducing sugar. In the other fluids there were some discrepancies between the estimates of total reducing sugar and glucose. However, these differences may be more apparent than real because with the limited amounts of these fluids available for assay, the values approached the limits of detection for the methods used. In fluid collected from the uterine horn, 50 mg/100 ml lactate was found. This is equivalent to approximately 5 mM lactate. In anoestrus ewes, and in ewes cannulated by other methods, somewhat lower levels of lactate were detected.

The protein concentration of the fluids varied from 1 to 3 g/100 ml but there were no significant variations due to the method of cannulating the uterus. The mean concentration of amino acids in the uterine fluids was 16 mM while that of urea was 4 mM. There were only minor variations between the fluids collected by the different techniques.

In the comparison of the organic constituents found in secretions collected from the uterine horn and fallopian tube of the same animal, the large differences recorded in the concentration of orcinol-reactive carbohydrate failed to reach the 5% level of significance in the overall statistical analysis. However, a paired *t*-test for the data from these animals showed significantly ($P < 0{\cdot}05$) higher levels of carbohydrate in the uterine fluid. There was also a higher concentration of protein in uterine than in tubal fluid ($P < 0{\cdot}05$).

(*d*) *Electrophoretic Analysis of Proteins in Uterine Fluids*

The electrophoretic mobilities of the various proteins in uterine fluid, relative to the albumin band, are shown in Table 4 together with the percentage of each protein in the fluids. The analyses of 11 samples of blood serum are also included in the table for comparison with the results from the uterine secretions. There were no significant differences either in relative mobilities of the various fractions or in the percentage of each protein fraction between the various uterine fluids analysed. Under the experimental conditions, the albumin band of uterine fluids migrated $43{\cdot}2 \pm 0{\cdot}9$ mm (mean $\pm$ standard error for 11 runs) and its mobility was identical with that of the albumin fraction of blood serum processed at the same time ($43{\cdot}0 \pm 1{\cdot}1$ mm). However, when the electrophoretic mobility of the other proteins of uterine fluid and blood serum were compared, it was found that the α_1-globulin of uterine fluid migrated at a faster rate than the α_1-globulin fraction of blood serum. In addition, albumin made a significantly lower contribution to the total soluble protein in uterine fluid than in blood serum while the opposite was true for β-globulin. In a comparison of the electrophoretic mobilities and the proportion of protein in uterine and tubal fluid from the same animal (Table 5) it was found that these fluids showed similar protein patterns.

(*e*) *Free Amino Acids in Uterine Fluids*

The contribution of individual amino acids to the total free amino acids of uterine fluids is shown in Table 6. For comparison, the amino acid composition of tubal fluid and blood serum are also included in the table. Alanine was the major neutral amino acid found in the uterine fluids and represented 20–30% of the total

TABLE 4

ELECTROPHORETIC MOBILITY OF AND PERCENTAGE OF PROTEINS IN UTERINE FLUIDS AND BLOOD SERA OF THE EWE

Technique of cannulation of uterus	Fluid analysed	Mobilities of globulins relative to albumin				Percentage of soluble protein in:				
						Albumin	Globulin			
		α_1	α_2	β	γ		α_1	α_2	β	γ
Via horn										
Unligated uterus										
Short cannula	Oestrous flow	0·85	0·66	0·43	0·23	48	4	9	32	7
Long cannula	Oestrous flow	0·83	0·67	0·41	0·22	59	2	9	27	4
Ligated uterus										
Short cannula	Oestrous flow	0·81	0·67	0·42	0·21	62	3	5	22	8
Long cannula	Oestrous flow	0·82	0·67	0·44	0·27	53	4	5	32	6
Via horn	Interoestrous pool	0·82	0·66	0·44	0·22	46	3	9	34	9
	Anoestrous pool	0·82	0·69	0·47	0·26	48	4	4	36	8
At uterocervical junction	Oestrous flow	0·85	0·69	0·46	0·24	57	4	6	26	7
Via cervical canal	Oestrous flow	0·85	0·69	0·48	0·25	42	7	6	35	9
Mean ± S.E.M. for all uterine fluids (27 samples)		0·83 ±0·004	0·67 ±0·005	0·46 ±0·007	0·24 ±0·006	52 ±1·7	4 ±0·6	7 ±0·6	31 ±1·4	7 ±0·6
Mean ± S.E.M. for blood serum (11 samples)		0·76 ±0·008	0·66 ±0·007	0·42 ±0·007	0·22 ±0·008	60 ±1·8	4 ±0·6	6 ±0·9	22 ±1·7	8 ±0·7

free amino acid content. Leucine and/or isoleucine, valine, and glycine were the other important neutral amino acids in all uterine fluids analysed. Two basic amino acids, lysine and arginine, were present in significant amounts, there being larger amounts of lysine than arginine. Glutamic acid was the major acidic amino acid identified and its

TABLE 5

COMPARISON OF THE ELECTROPHORETIC PATTERN OF UTERINE AND OVIDUCAL FLUIDS FROM THE SAME ANIMALS

Protein	Mobility relative to albumin		Percentage of soluble protein in respective fractions	
	Fallopian tube	Uterus	Fallopian tube	Uterus
Albumin	1·00	1·00	48	41
Globulin α_1	0·84	0·87	4	5
α_2	0·69	0·70	8	5
β	0·43	0·48	30	38
γ	0·26	0·24	10	11

TABLE 6

MAJOR FREE AMINO ACIDS IN UTERINE FLUID, OVIDUCAL FLUID, AND BLOOD SERUM (PERCENTAGE OF TOTAL NINHYDRIN-REACTIVE COMPOUNDS)

Values are means ± S.E.M. The number of samples analysed is given in parentheses at the head of each column. n.d., not detected

Amino acid identified	Uterine fluid					Tubal fluid (6)	Blood serum (3)
	Oestrous fluid* (12)	Intercycle fluid* (3)	Anoestrous fluid* (3)	Oestrous fluid† (4)	Oestrous fluid‡ (3)		
Alanine	20±1	30±5	23±3	17±2	26±6	22±3	13±1
Arginine	5±1	6±3	7±1	8±6	9±3	n.d.	5±1
Aspartic acid	4±1	2±1	2±1	3±4	2±1	n.d.	1±0
Glutamic acid	10±1	2±2	3±1	13±5	6±2	5±1	9±1
Glutamine	n.d.	n.d.	n.d.	n.d.	n.d.	3±0	13±2
Glycine	8±2	15±3	7±2	11±4	12±2	23±4	13±1
Histidine	trace	trace	trace	trace	trace	5±1	5±1
Leucine and/or isoleucine	23±2	13±2	24±1	22±4	14±2	8±2	8±0
Lysine	14±1	23±3	13±1	12±2	16±3	9±1	10±2
Phenylalanine	n.d.	n.d.	n.d.	n.d.	n.d.	4±0	*c.* 1%
Proline	present	present	present	present	present	present	present
Serine	n.d.	n.d.	n.d.	n.d.	n.d.	3±0	4±0
Valine	16±1	9±2	21±1	15±3	16±4	9±1	8±0
Total amino acids (mM)	16±4	15±2	17±3	9±1	23±2	10±1	5±1

* Horn cannulation.
† Uterocervical cannulation.
‡ Cannulation via cervix.

contribution to the total amino acid content showed considerable variation between times of collection. At oestrus, this amino acid represented 6–12% of the total amino acids of the fluid. Fluid collected during the interoestrous period and fluid from anoestrous ewes contained a smaller proportion of this amino acid. Small amounts of aspartic acid were also present.

Like uterine fluid, blood serum contained alanine, glycine, leucine and/or isoleucine, valine, lysine, arginine, and glutamic acid as the major amino acids. However, the concentration of total amino acids in blood serum (5 mM) was less than half that in uterine fluid and as a result the concentration of the individual amino acids in serum was lower than in uterine fluid. In addition, blood serum contained small amounts of serine, histidine, and the amine glutamine, which were not identified in the uterine fluid. In tubal fluid alanine and glycine made up 45% of the total amino acids and their concentrations were similar to those in uterine fluid. Serine, histidine, and glutamine were also present in tubal fluid but no arginine and aspartic acid were found.

IV. Discussion

In the present study, cannulation of the sheep uterus via the cervical canal resulted in the collection of fluid with a substantially different chemical composition from that collected by the other two methods. Cannulation via the cervical canal has been found least satisfactory for the collection of uterine fluids (Wales and Restall 1971); success rate was low and the secretions collected showed evidence of leucocyte invasion, presumably due to the increased susceptibility to contamination from the exterior with this method of cannulation. Such contamination could well be expected to influence the chemical composition of the fluid collected.

Differences in the chemical composition between fluid collected via the uterine horn and that collected from the body of the uterus following transection anterior to the cervix were less marked. The latter method of cannulation resulted in collection of a secretion with an osmotic pressure approximately 20% lower than that of fluid collected by the other method, due mainly to a reduction in the concentration of sodium and chloride ions. These results suggest that a possible dilution of the fluid occurs during collection. This could result from a direct effect of the cannula on the mucosa since this method of cannulation, as distinct from cannulation via the horn, reduced the length of the interoestrous period.

In the analysis of inorganic ions present in the fluids, close agreement was found between the ionic equivalents and the values expected from the estimates of osmotic pressure based on freezing point depression. These findings indicate that the majority of the osmotically active constituents had been measured and that any such unidentified compounds must be present in low concentration.

The ionic composition of uterine fluid recorded in the present study is similar to that for tubal fluid of the ewe and differs from blood plasma of similar ewes in having a higher concentration of potassium and a lower concentration of calcium and bicarbonate (see Restall and Wales 1966). Howard and DeFeo (1959) have also recorded higher concentrations of potassium in rat uterine fluid than in serum. The ratio of sodium to potassium found in the present study averaged 15 : 1 and never fell below 8 : 1. These values agree well with those recorded by Heap (1962) for the luminal fluid of ewes collected at oestrus and those recorded by Iritani *et al.* (1971) for uterine fluid collected from the cannulated rabbit uterus. In many species, including the ewe during the luteal phase, Na : K ratios as low as 2–3 : 1 have been recorded for uterine fluid collected by a variety of methods not involving cannulation (Olds and Van Demark 1957; Howard and DeFeo 1959; Ringler 1961; Heap 1962). It is difficult to give a satisfactory explanation for these low Na : K ratios. In normal

circumstances potassium is confined mainly to the cytosol and low Na : K ratios would not be expected in the uterine lumen unless considerable cytolysis occurred at some stage in the cycle. Comparison with present results is made difficult by the fact that the authors quoted above made no satisfactory account of total acid and alkaline equivalents and in those cases where chloride, as well as sodium and potassium, was measured, large discrepancies existed between anionic and cationic balance of these major ions.

The concentrations of protein and glucose in uterine fluid were lower than those found in plasma (Long 1961) and the major energy substrate identified was lactic acid. Iritani *et al.* (1969) found similar concentrations of lactate but more elevated levels of glucose. The relatively high concentrations of free amino acids present in the uterine secretions could well be important sources of nutrients either for the gametes or for the developing zygote prior to and during implantation. In the mouse, arginine, histidine, leucine, threonine, and cystine are necessary for trophoblastic outgrowth of the blastocyst *in vitro* (Gwatkin 1966). Three of these amino acids were identified in the uterine secretion of the ewe. However, without knowing more of the specific requirements for amino nitrogen during implantation in this species, it would be dangerous to speculate on the physiological significance of these findings.

The protein pattern found in the luminal fluid of the sheep uterus showed differences both in electrophoretic mobility and relative concentration from those found in blood serum. Using various methods of electrophoresis, other authors have noted the presence of protein components in the uterine fluid of various species which are not present in plasma (Ringler 1961; Stevens *et al.* 1964; Urzua *et al.* 1970). These findings, plus the fact that the concentration of other constituents such as amino acids, glucose, and several inorganic ions differ between blood and uterine secretion, support the earlier suggestion (Fahning *et al.* 1967) that uterine fluid is, at least in part, an active secretion of the uterine glands and not merely a blood transudate.

Iritani *et al.* (1969) found cyclic variations in the concentration of some uterine constituents following cannulation. In the present experiment, variations in the concentration of orcinol-reactive carbohydrate and some amino acids were found between fluids collected during the elevated secretion associated with behavioural oestrus and those collected between each oestrous flow. High concentrations of glutamic acid, leucine and/or isoleucine, and valine were found at oestrus while the opposite was true for alanine, glycine, and lysine. However, in making comparisons between fluids collected by cannulation at these two periods, it must be remembered that secretion rates as low as 0·1–0·2 ml/24 hr are recorded in the interval between oestrous periods. The volume of fluid contained in the bore of the cannula is approximately 0·8 ml and thus, during times of low secretion, fluid can be retained in the cannula for several days prior to entering the collection bottle and may undergo chemical change. At the time of oestrus, when sojourn in the cannula is considerably decreased, the chemical composition of the fluid collected probably more closely approaches that produced by the uterus.

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VI. References

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