THE FALLOPIAN TUBE OF THE SHEEP

III. THE CHEMICAL COMPOSITION OF THE FLUID FROM THE FALLOPIAN TUBE

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Summary

The chemical composition of fluid from the fallopian tube of the ewe was studied in four cycling ewes and in four groups of four ovariectomized ewes receiving oestrogen and progesterone in factorial combination. Both the major electrolytes and some organic constituents in the fluids were examined.

Sodium was found to be the main cation present and chloride the main anion. Smaller amounts of potassium, magnesium, calcium, phosphate, and bicarbonate were also present. Fluid from the normal ewes contained less magnesium than that from the spayed ewes. Within the spayed ewes, oestrogen caused an increase in potassium and bicarbonate and a decrease in magnesium. The concentration of sodium, potassium, chloride, and bicarbonate were lowest during metoestrus. The concentration of magnesium, on the other hand, was lowest during oestrus and highest during dioestrus.

Total phosphorus, acid-insoluble phosphorus, total orcinol-reactive carbohydrate, protein, and lactic acid in the fluids was also assayed. All these constituents showed considerable variation between samples. However, no changes during the oestrous cycle were evident. Glucose could not be detected in any of the fluids.

Within the spayed ewes, oestrogen, administered alone, decreased the concentration of protein but when given in combination with progesterone, caused the opposite effect. Carbohydrate and lactate increased during the second cycle of treatment in those spayed ewes receiving oestrogen. Acid-insoluble phosphorus was found to increase in the second cycle of treatment in all animals.

I. Introduction

It has been suggested by many investigators (see Van Demark 1958; Bishop 1961) that the lumenal fluids of the female genitalia serve as a vehicle and nutrient media for the gametes. Other studies suggest that the fluids may influence the activity of the spermatozoa (Olds and Van Demark 1957*c*) and the nutrition of the blastocyst (Lutwak-Mann 1959). It is also possible that these fluids exert as yet unknown influences on the physiology of the gametes, and the phenomenon termed capacitation (Austin 1951; Chang 1951) is of interest in this regard. While there is much work reported on the enzymology and chemical composition of the tissues of the

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reproductive tract [see reviews by Olds and Van Demark (1957*a*) and Gross (1961)], there is little in the literature on the chemical composition of the lumenal fluids of the female genitalia, especially the ewe.

This paper presents data on the chemical composition of the tubal fluid of the ewe and its hormonal control.

II. Materials and Methods

(*a*) *General*

Twenty ewes with cannulae in their fallopian tubes were treated as follows during each 17-day cycle period:

(1) Four spayed ewes were given 10 mg/day intramuscularly of progesterone on days 2–13 inclusive, followed by 30 μg intramuscularly of oestradiol benzoate on day 15.

(2) Four spayed ewes were given 30 μg intramuscularly of oestradiol benzoate on day 15.

(3) Four spayed ewes were given 10 mg/day intramuscularly of progesterone on days 2–13 inclusive.

(4) Four spayed ewes were given no treatment.

(5) Four normal cycling ewes received no treatment; day 1 of the cycle of these ewes was taken as the day on which oestrus was first detected.

The ewes received one cycle of treatment before observations commenced on two subsequent cycles.

The 17-day treatment cycle was arbitrarily divided into three periods henceforth referred to as stage 1 (day 1–2), stage 2 (days 3–7), and stage 3 (days 8–16). In the normal cycling ewes in which the oestrous cycle exceeded 17 days, stage 3 was extended. In the spayed ewes receiving oestradiol benzoate and in the normal ewes the three stages correspond approximately to the physiological states of oestrus (stage 1), metoestrus (stage 2), and dioestrus (stage 3). In the other two groups of ewes, in which a cyclic pattern was not expected, the division into stages is purely for comparison. This experimental design has been discussed in detail by Restall (1966).

Tubal fluid from each ewe was collected daily and the collections were grouped into the three stages defined above. The pooled fluids were frozen at −30°C until chemical analyses could be performed.

(*b*) *Analytical Procedures*

Sodium, potassium, calcium, and magnesium were estimated with an atomic absorption spectrophotometer (Davey 1963). Chloride and phosphorus were determined colorimetrically (Allen 1940; Schoenfeld and Lewellan 1964). Acid-insoluble phosphorus was estimated after precipitation with 20% (w/v) trichloroacetic acid. Protein was determined by the biuret method (Wales, Scott, and White 1961); orcinol-reactive carbohydrate by the method of Hewitt (1937); lactic acid by the lactic dehydrogenase method of Barker and Britton (1957); and bicarbonate by adding a known excess of hydrochloric acid and back-titrating with sodium hydroxide (Van Slyke 1922). Glucose was determined enzymatically by the method of Huggett and Nixon (1957).

Table 1

CONCENTRATIONS OF ELECTROLYTES IN TUBAL FLUID FROM NORMAL EWES AND FROM SPAYED EWES RECEIVING PROGESTERONE (P) AND OESTRADIOL BENZOATE (ODB)

Results are for the first 17-day cycle of treatment

Ewes	Hormone		Stage of Cycle	Electrolyte Concentration (m-equiv/l)						
	P	ODB		Na^+	K^+	Ca^{2+}	Mg^{2+}	PO_4^{3-}	Cl^-	HCO_3^-
Normal	—	—	1	136	8·24	2·72	0·71	0·98	119	21·8
			2	128	8·38	2·74	0·67	0·75	112	15·6
			3	146	7·84	3·04	0·88	1·59	133	21·8
Mean*				137	8·16	2·84	0·75	1·11	121	19·7
Spayed	+	+	1	145	8·41	2·44	0·79	1·51	129	21·2
			2	129	7·75	3·22	1·02	1·75	127	17·2
			3	143	8·34	2·85	0·79	1·68	132	17·0
Mean				139	8·17	2·84	0·87	1·65	129	18·5
Spayed	—	+	1	143	8·86	2·17	0·65	1·21	137	28·5
			2	129	7·75	2·65	0·84	1·20	131	20·0
			3	139	7·94	1·88	0·74	1·18	132	20·1
Mean				137	8·12	2·24	0·74	1·19	133	22·9
Spayed	+	—	1	135	7·46	3·44	1·23	1·79	136	17·0
			2	138	7·09	2·95	1·04	1·90	129	16·8
			3	143	7·52	3·31	1·44	1·74	134	18·4
Mean				139	7·36	3·24	1·24	1·81	133	17·4
Spayed	—	—	1	136	8·20	4·44	1·08	1·73	136	14·3
			2	129	7·55	2·58	1·06	1·57	116	17·1
			3	133	8·50	3·27	1·67	1·59	138	18·2
Mean				133	8·09	3·43	1·27	1·63	130	16·5

* Means calculated from original data.

In order to show the relative magnitudes and interrelations of the electrolytes, their values have been expressed in milliequivalents per litre (see Gamble 1954). In these calculations the valency of the phosphate ion has been taken as 1·8 (Gamble 1954).

(c) *Statistical Analyses*

The pooled fluid samples were classified according to the type of hormone treatment given to the ewe, the stage of the cycle, and the cycle of treatment. Because of the small amounts of fluid available and the multiplicity of assay procedures employed, it was not possible to perform all the analyses on any one pooled sample and therefore complete data within ewes was unobtainable. Analyses of variance were carried out for each chemical constituent using the method of unweighted means for disproportionate subclass numbers (Snedecor 1957). An error

TABLE 2

CONCENTRATIONS OF ELECTROLYTES IN TUBAL FLUID FROM NORMAL EWES AND FROM SPAYED EWES RECEIVING PROGESTERONE (P) AND OESTRADIOL BENZOATE (ODB)

Results are for the second cycle of treatment

Ewes	Hormone		Stage of Cycle	Electrolyte Concentration (m-equiv/l)						
	P	ODB		Na^+	K^+	Ca^{2+}	Mg^{2+}	PO_4^{3-}	Cl^-	HCO_3^-
Normal	–	–	1	138	9·09	3·09	0·58	1·02	126	22·6
			2	123	7·49	3·81	0·74	1·38	139	17·0
			3	136	8·99	3·32	1·04	1·38	127	17·6
Mean*				132	8·53	3·41	0·79	1·26	131	19·1
Spayed	+	+	1	143	7·91	2·78	0·78	1·91	126	27·5
			2	139	7·29	2·98	0·81	1·19	131	16·0
			3	141	8·50	3·02	1·04	1·66	141	15·3
Mean				141	7·90	2·93	0·88	1·59	133	19·6
Spayed	–	+	1	139	9·79	3·88	0·72	1·25	134	21·3
			2	126	8·24	2·90	0·91	1·05	129	19·1
			3	130	8·21	3·07	1·02	1·39	131	20·0
Mean				132	8·42	3·29	0·88	1·23	131	20·1
Spayed	+	–	1	133	7·29	3·32	0·90	1·66	126	13·0
			2	128	7·16	1·71	0·87	1·30	115	13·1
			3	131	8·01	3·24	1·31	1·92	138	13·9
Mean				131	7·49	2·76	1·03	1·63	126	13·3
Spayed	–	–	1	141	7·62	2·15	0·75	1·94	127	15·6
			2	123	6·89	2·83	0·71	2·03	124	13·0
			3	141	7·29	2·98	0·97	1·83	139	16·0
Mean				135	7·27	2·65	0·81	1·93	130	14·9

* Means calculated from original data.

mean square was calculated from the individual estimations within treatments for each analysis. Because of the dependency of stage of cycle upon treatment, these two effects were tested against the appropriate interaction mean squares.

Within the treatments, differences between the means for each cycle were tested by *t*-tests, using a standard error calculated from the appropriate interaction mean square in the analysis of variance. Where appropriate, significant effects with more than one degree of freedom have been partitioned using orthogonal polynomial coefficients.

III. Results

The data from the electrolyte determinations are shown in Tables 1 and 2 with a summary of the analyses of variance in Table 3. In the tubal fluid from the normal ewes, sodium was found to be the major cation and chloride the major anion. The mean levels (in milliequivalents per litre) of the electrolytes from these ewes were: sodium 135; potassium 8·35; calcium 3·13; magnesium 0·77; phosphate 1·19; chloride 126; and bicarbonate 19·4. There were no significant differences between the means for each cycle of treatment in any of the constituents. However, there was a significant difference between stages of the cycle, and in general lowest levels of the constituents were observed in stage 2 fluids.

Table 3

SUMMARY OF ANALYSES OF VARIANCE OF DATA IN TABLES 1 AND 2

Variance ratios are given except where indicated

Source of Variation	Degrees of Freedom	Na^+	K^+	Ca^{2+}	Mg^{2+}	PO_4^{3-}	Cl^-	HCO_3^-
Main effects								
Treatment								
Normal *v.* rest	1	0·21	2·51	0·73	7·40*	2·62	1·91	1·16
Within spayed ewes								
Effect of ODB	1	1·58	4·72*	0·89	14·40**	2·31	0·31	14·49*
Effect of P	1	1·96	0·78	0·03	1·36	0·59	0·08	1·27
ODB × P	1	1·01	0·00	0·16	0·00	1·12	0·03	0·73
Stage of cycle	2	9·45**	2·78*	0·44	8·40**	0·30	3·57*	3·98*
Cycle of treatment	1	1·76	0·05	0·23	2·80	0·01	0·11	2·07
Interactions								
Treatment × cycle								
Normal *v.* rest	1	0·26	0·36	1·64	1·32	0·07	2·30	0·18
Within spayed ewes								
Effect of ODB	1	0·09	0·42	8·11**	10·16**	0·02	0·47	0·69
Effect of P	1	0·07	0·02	0·62	0·22	0·44	0·00	0·09
ODB × P	1	3·38*	1·86	2·25	2·16	0·20	0·99	1·59
Other interactions	18	0·62	0·32	1·16	0·73	0·17	0·69	0·84
Between estimations (mean squares)	30	65·18	0·92	0·53	0·05	0·29†	61·88‡	9·28§

* $P<0·05$. ** $P<0·01$.

† 27 degrees of freedom. ‡ 22 degrees of freedom. § 20 degrees of freedom.

The electrolyte composition of fluid from the spayed ewes was similar to that from normal ewes except that the concentration of magnesium in normal ewes (0·77 m-equiv/l) was significantly lower than that in spayed ewes (0·96 m-equiv/l). Within the spayed ewes, however, oestrogen was observed to have a significant effect on the levels of potassium, magnesium, and bicarbonate. Under the influence of oestrogen, concentrations of potassium and bicarbonate were increased while magnesium was decreased. In those ewes receiving oestrogen the concentration of magnesium and calcium increased during the second cycle of treatment.

Considering the normal and spayed ewes together, there were significant differences in the concentrations of sodium, potassium, magnesium, chloride, and bicarbonate between the stages of the cycle (Table 4). With the exception of magnesium the lowest levels of these constituents were found in stage 2 of the cycle. Magnesium increased in concentration from stage 1 to stage 3 of the cycle.

TABLE 4

MEAN LEVELS OF ELECTROLYTES FOR EACH STAGE OF THE TREATMENT CYCLES IN ALL EWES

Stage of Cycle	Electrolyte Level (m-equiv/l)						
	Na^+	K^+	Ca^{2+}	Mg^{2+}	PO_4^{3-}	Cl^-	HCO_3^-
1	138·9	8·19	3·04	0·82	1·50	129·5	20·4
2	128·9	7·54	2·84	0·87	1·34	125·2	16·5
3	138·2	8·12	3·00	1·09	1·60	134·5	17·8

The acid and alkaline equivalents for each group of ewes are set out in Table 5, together with those obtained for sheep plasma. In all ewes sodium was the major cation and chloride the major anion. There was good agreement between the acid and alkaline equivalents in all groups but there were differences from blood in the levels of sodium, potassium, calcium, magnesium, chloride, and bicarbonate.

TABLE 5

ACID AND ALKALINE EQUIVALENTS IN BLOOD PLASMA, IN TUBAL FLUID FROM NORMAL AND SPAYED EWES, AND IN TUBAL FLUID FROM SPAYED EWES RECEIVING HORMONE TREATMENTS

Fluid	Acid or Alkaline Equivalent of Electrolyte (m-equiv/l)								
	Na^+	K^+	Ca^{2+}	Mg^{2+}	Total	Cl^-	HCO_3^-	PO_4^{3-}	Total
Blood plasma	141	1·38	5·69	1·87	149·9	111	26·2	3·61	140·8
Tubal fluid from normal ewes	135	8·40	3·13	0·77	147·3	126	19·4	1·19	146·6
Tubal fluid from spayed ewes:									
Treated with progesterone + oestradiol benzoate	140	8·03	2·89	0·87	151·8	131	19·0	1·62	151·6
Treated with oestradiol benzoate alone	134	8·27	2·76	0·81	145·0	132	21·5	1·21	154·7
Treated with progesterone alone	135	7·42	2·99	1·13	146·5	130	15·4	1·72	147·3
No hormone treatment	134	7·68	3·05	1·04	145·8	130	15·7	1·78	147·5

The results for the estimation of other chemical constituents are given in Tables 6 and 7 with a summary of the analyses of variance in Table 8. For the normal ewes the mean concentrations of the various chemical components were: total phosphorus 2·94 mg/100 ml, acid-insoluble phosphorus 0·97 mg/100 ml, total orcinol-reactive carbohydrate 60·9 mg/100 ml, protein 1·36 g/100 ml, and lactate 3·32 μmoles/ml. There was considerable variation between individual estimations but there were no significant differences between the means for each cycle of treatment and there were no differences between stages of the cycle. No attempt was made to balance the levels of total phosphorus with acid-insoluble and acid-soluble phosphorus as it was not possible to carry out all these estimations on the same sample.

TABLE 6

CONCENTRATIONS OF SOME CHEMICAL CONSTITUENTS OF TUBAL FLUID FROM NORMAL EWES AND FROM SPAYED EWES RECEIVING PROGESTERONE (P) AND OESTRADIOL BENZOATE (ODB)

Results are for the first cycle of treatment

Ewes	Hormone		Stage of Cycle	Total Phosphorus (mg/100 ml)	Acid-insoluble Phosphorus (mg/100 ml)	Total Carbo-hydrate (mg/100 ml)	Protein (g/100 ml)	Lactate (μmoles/ml)
	P	ODB						
Normal	—	—	1	4·05	0·87	69·4	1·45	3·91
			2	2·13	0·46	55·0	1·60	3·68
			3	4·63	0·84	69·1	1·59	3·44
Mean*				3·60	0·72	64·5	1·55	3·68
Spayed	+	+	1	2·50	2·06	97·9	4·46	5·85
			2	1·27	1·93	51·6	1·94	4·15
			3	4·64	1·36	52·5	2·73	4·84
Mean				2·80	1·78	67·3	3·04	4·95
Spayed	—	+	1	4·34	0·56	64·7	1·74	1·54
			2	2·24	0·45	46·8	0·63	1·72
			3	1·55	0·68	62·1	1·92	1·07
Mean				2·71	0·56	57·5	1·43	1·11
Spayed	+	—	1	4·28	1·13	82·9	2·71	2·97
			2	5·39	1·31	69·7	2·66	3·02
			3	1·97	1·16	68·4	2·29	1·70
Mean				3·88	1·20	73·7	2·55	2·56
Spayed	—	—	1	3·73	0·77	97·8	2·78	4·41
			2	2·44	0·42	69·4	3·44	3·90
			3	2·92	1·02	96·9	2·96	4·00
Mean				3·03	0·73	88·0	2·96	4·10

* Means are calculated from original data.

Fluid from spayed ewes was similar to that collected from normal ewes except that higher protein concentrations were found in the spayed ewes. Within the spayed ewes, fluid from animals given oestrogen alone contained very little protein. When oestrogen was given in combination with progesterone, the level of protein was similar to that recorded in ewes not receiving oestrogen. The concentration of carbohydrate increased from cycle 1 to cycle 2 in fluid from spayed ewes receiving oestrogen and progesterone alone. In the other spayed ewes, the concentration of carbohydrate declined between cycles. In the first cycle, the minimum concentration of carbohydrate occurred during stage 2, and in the second cycle the level of carbohydrate was maximum during this stage. The level of acid-insoluble phosphorus was lower in the first cycle (1·00 mg/100 ml) than in the second cycle (1·54 mg/100 ml).

Table 7

Concentrations of some chemical constituents of tubal fluid from normal ewes and from spayed ewes receiving progesterone (P) and oestradiol benzoate (ODB)

Results are for the second cycle of treatment

Ewes	Hormone		Stage of Cycle	Total Phosphorus (mg/100 ml)	Acid-insoluble Phosphorus (mg/100 ml)	Total Carbohydrate (mg/100 ml)	Protein (g/100 ml)	Lactate (μmoles/ml)
	P	ODB						
Normal	–	–	1	2·28	0·77	61·2	0·93	2·15
			2	1·50	0·80	43·7	0·48	1·54
			3	3·04	2·07	67·0	2·10	5·17
Mean				2·27	1·21	57·3	1·17	2·95
Spayed	+	+	1	2·75	1·86	77·5	2·90	2·22
			2	0·33	1·25	124·2	3·36	1·33
			3	3·04	2·10	113·8	2·39	1·84
Mean				2·04	1·73	105·2	2·88	1·79
Spayed	–	+	1	1·92	2·29	67·3	1·26	4·13
			2	1·04	1·51	60·1	1·08	3·04
			3	5·06	1·13	65·0	0·41	2·83
Mean				2·67	1·64	64·1	0·92	3·33
Spayed	+	–	1	3·20	1·20	80·0	2·70	1·15
			2	3·35	1·41	77·4	1·69	0·58
			3	4·29	2·33	69·6	2·84	0·44
Mean				3·61	1·65	75·7	2·74	0·72
Spayed	–	–	1	2·20	1·20	90·0	2·70	1·26
			2	3·67	1·39	94·5	3·55	0·89
			3	0·49	1·86	64·1	1·53	0·74
Mean				2·12	1·48	82·9	2·59	0·96

Although no glucose was detected in any of the tubal fluids examined, there were significant amounts of lactate present in all fluids. The concentration of lactate in those ewes receiving oestrogen increased in the second cycle. However, in those ewes receiving progesterone, the level of lactate declined in the second cycle.

TABLE 8

SUMMARY OF THE ANALYSES OF VARIANCE ON THE DATA IN TABLES 6 AND 7

Variance ratios are given except where indicated

Source of Variation	Degrees of Freedom	Total Phosphorus	Acid-insoluble Phosphorus	Total Carbohydrate	Protein	Lactate
Main effects						
Treatment						
Normal *v.* rest	1	0·02	1·37	3·34	12·00**	0·49
Within spayed ewes						
Effect of ODB	1	1·30	0·52	0·30	6·16*	0·40
Effect of P	1	0·72	2·78	0·52	8·93**	0·01
ODB × P	1	1·84	0·35	8·72	13·30**	0·84
Stage of cycle	2	0·99	0·65	0·46	0·53	2·42
Cycle of treatment	1	1·19	4·36*	0·66	1·21	1·77
Interactions						
Treatment × cycle						
Normal *v.* rest	1	0·50	0·01	2·53	0·05	1·45
Within spayed ewes						
Effect of ODB	1	0·03	0·02	5·86*	0·14	14·07**
Effect of P	1	0·00	1·50	4·30*	0·88	13·30**
ODB × P	1	0·42	0·50	1·56	0·08	35·71**
Stage × cycle	2	0·68	0·44	3·68*	0·86	1·09
Other interactions	16	1·26	0·33	1·30	1·12	1·23
Between estimations (mean squares)		1·69 (22 d.f.)	0·51 (26 d.f.)	122·37 (32 d.f.)	0·44 (45 d.f.)	0·47 (28 d.f.)

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$.

IV. Discussion

The results show that the electrolyte composition of tubal fluid collected from the normal ewes differs from that of blood. Levels of potassium are approximately four times as high as blood, those of chloride 30% higher, and those of calcium, magnesium, and sodium generally lower. Howard and de Feo (1959) noted a similar elevation in the concentration of potassium in the rat uterus and concluded that a true secretion occurred. Ringler (1961), also studying the uterine fluid of the rat, found levels of potassium 10 times greater than found in blood and concluded that this fluid was at least supplemented by a true secretion. The lowest concentrations of the electrolytes in the normal ewes occurred during metoestrus, when endogenous levels of oestrogen can be expected to be waning. The other chemical constituents of this fluid did not show any apparent cyclic effect although there was considerable individual variation.

The concentration of lactate in the fluids could provide a substrate for the metabolism of spermatozoa. The source of lactic acid is probably the epithelium itself. Mastroianni, Winternitz, and Lowi (1958) demonstrated that the human endosalpinx preferentially utilizes the glycolytic pathway of metabolism. No glucose was detected in the fluid but another possible substrate could be phospholipid, which may be present in the acid-insoluble phosphorus fraction. Levels of carbohydrate and protein are high and probably derived largely from the mucoprotein which has been demonstrated histochemically in tubal fluid from the sheep (Hadek 1953). The absence of a significant difference between cycles in any of the elements studied in fluid from the entire ewes would indicate that the methods of fluid collection employed here are suitable for relatively long-term studies.

The results from the spayed ewes show clearly that some of the chemical constituents of tubal fluid are under endocrine control, and the cyclic variation in the ewes receiving oestrogen is similar to that observed in the normal ewes. The rise in bicarbonate concentration caused by oestrogen may be related to the effects of this hormone on carbonic anhydrase in the female reproductive tract (Lutwak-Mann 1955; Bialy and Pincus 1962). The finding that the levels of magnesium in the normal ewes differ significantly from those in the spayed ewes may indicate that some other factor may enter into the control of its concentration.

With regard to the other chemical constituents examined in the spayed ewes, total carbohydrate, protein, and lactate were influenced by the hormones administered. Although there were no significant differences in the concentration of protein between stages of the cycle in the present study, other workers have found cyclical changes in the level of protein in fluids from other parts of the female genital tract. Thus Ringler (1961) found that oestrogen elevated the levels of protein in uterine fluid from the rat, and Wallace, Stone, and White (1965) found a greater protein concentration in rinsings from the rat uterus during pro-oestrus and oestrus.

Because of the varying methods that have been used to recover genital fluids for study, comparisons of the present results with other published reports must be made cautiously. Heap (1962), using uterine rinsings from the rat, rabbit, sheep, and cow, has found species differences and also noted that, in the sheep, the levels of sodium, potassium, chloride, nitrogen, and carbohydrate were greater during the luteal phase of the cycle. Olds and Van Demark (1957*b*) studied various lumenal fluids from the genitalia of slaughtered cows and found distinct differences between fluids. Although using small quantities of fluid, these authors were able to demonstrate an apparent difference in the concentrations of sodium and potassium between stages of the oestrous cycle in different fluids.

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