D-tartaric acids, glycine and the sodium salt of L- α -hydroxy- β -sulphopropionic acid.

2. Thiocyanate and the sodium salt of acetylenedicarboxylic acid were found to be non-competitive inhibitors.

3. The following compounds were found to be without effect on fumarase: the sodium salts of acetic, butyric, crotonic, L- and DL-aspartic, and acetoacetic acids, the mono- and di-methyl esters of fumarate, and the SH inhibitors, iodoacetate, iodoacetamide, iodosobenzoate, *p*-chloromercuribenzoate and chloroacetophenone.

4. The inhibition of all the competitive inhibitors was dependent on the temperature. Of the three inhibitors investigated in detail at low temperatures the combination of fumarase and inhibitors was found to be exothermic, while at higher temperatures the combination was found to be endothermic.

5. Inhibition by all the competitive inhibitors tested was markedly dependent on pH. The variation of the inhibitor constants and of the Michaelis constants with pH has been studied in detail, and the results are discussed in the light of the theory of Dixon (1953).

6. A tentative hypothesis of the structure of the active centre and the sequence of reactions which may accompany the reversible hydration of fumarate to L-malate is suggested on the basis of these results.

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Absorption of Immune Globulin by the Young Lamb after Ingestion of Colostrum

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The role of colostrum in promoting the well being of the young ruminant has been established mainly by work on the cow. It has been shown that these animals depend on the ingestion of colostrum soon after birth and the absorption of immune globulins from this secretion for a passive immunity which greatly favours their chances of survival of the critical first few weeks of their life (Smith & Little, 1922; Orcutt & Howe, 1922). That a similar situation obtains in the sheep has been shown in three different ways; the immunological studies of Mason, Dalling & Gordon (1930) and Schnieder & Szathmary (1939); the work on the sodium sulphate salted-out protein fractions of Earle (1935) and the electrophoretic examinations of Charlwood & Thomson (1948). In the present work these three methods have been combined in a study of the changes in the serum proteins of suckling lambs and

extended to a series of lambs receiving colostrum after various periods of delay. In relation to these changes, the existence and nature of any proteinuria in the young lambs has been investigated.

METHODS

Salted-out nitrogen fractions. Serum protein, globulin and albumin N fractions were estimated by difference from the total N, 'globulin-free' N and non-protein N contents; these were determined by micro-Kjeldahl analysis of the following solutions representing a dilution of 1 in 21 of the serum sample: a dilution in normal saline, a globulin-free filtrate obtained after precipitation with saturated $MgSO_4$ at 20° overnight, and a protein-free filtrate obtained after precipitation with trichloroacetic acid (5 g./100 ml.). Colostrum N fractions were obtained by analysing a suitable dilution in saline in the same way as serum with the following addition: casein N was obtained by including the analysis of a casein-free filtrate prepared by acidification of a dilution in saline with acetic acid.

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Electrophoretic examination of the sera. This was carried out in the Tiselius apparatus at 4° using phosphate buffer at pH 8.0 and ionic strength 0.2 and a protein concentration of about 2 g./100 ml. The percentage composition was determined from the diagrams by projection, extrapolation and planimetry. As considerable difficulty was experienced in analysing the individual components, the method of extrapolation employed by Dalgarno, Godden & McCarthy (1950) was used throughout. The albumin and salt boundaries were completed. The slowest component, probably corresponding to the γ -globulin of human serum was then extrapolated and called component 2. The area between this component and the albumin was not subjected to further analysis but was measured as a single component and termed component 1.

Antibodies. Antibodies in colostrum, serum and urine were estimated by the limiting dilution of the agglutinin test. At first the tests were carried out from a lower limit of 1 in 25, but later, samples which were negative at this limit were examined on a lower range from a dilution of 1 in 2.

Urine examination. This consisted of qualitative tests for protein by sulphosalicylic acid precipitation and by heat coagulation (with addition of acetic acid). Globulin was detected by precipitation with half-saturated $(\text{NH}_4)_2\text{SO}_4$ and with saturated MgSO_4 . These tests were made comparative by an arbitrary grading of the degree of precipitation as a trace, +, ++ and +++. Antibodies were tested for in the lower range of dilutions, that is from a dilution of 1 in 2.

RESULTS

The present observations were made on Cheviot, Blackface and half-bred lambs over a period of two lambing seasons. The ewes which gave birth to them were immunized with *Salmonella typhosa* antigen. The first injection was given 21 days before the expected lambing date. After birth, some lambs were allowed to suck the ewe in the normal way, some were bottle-fed with cows' milk for varying periods before colostrum feeding, while others never received colostrum. The lambs which received

colostrum after a delay, did so, either by returning to the unsucked dam when the delay was short, or by being fostered on to another ewe which had recently lambed but not suckled, or by ingesting a single pint of stored colostrum. The relations and treatments of these animals are given in Table 1.

Observations were made on 15 ewes and 17 lambs. Seven lambs ingested colostrum at birth, six after delays of 12, 24, 29 and 48 hr. and 3 and 9 days, respectively, and four were deprived of colostrum.

The serum and colostrum of the ewes at lambing were examined for salted-out nitrogen fractions and antibodies. In two ewes, the colostrum was further examined at various intervals after lambing. In two other ewes it was also examined before lambing took place. The results presented for the ewes, Table 2, have been limited to thirteen relating to those given for the lambs, Table 3.

The lambs' serum was also examined by these two methods and in addition by electrophoretic analysis. Samples were taken at the following times: birth, 2-3 days, 7-10 days and at 5-6 weeks after birth. The results, Table 3, in the case of lambs ingesting colostrum at birth, have been limited to four typical cases. The urine of twelve lambs was examined for a few days after birth and ingestion of colostrum, Table 4.

The composition of colostrum, Table 2, showed at lambing protein nitrogen ranging from approximately 1 to 4 g./100 ml. and titres from 1 in 1000 to 1 in more than 10 000. The subsequent rapid change in composition is illustrated by the data included for ewes 232 and 255, Table 2.

The observations on colostrum before lambing were made on ewes 13 and 22, Table 2. The colostrum of ewe 13 obtained 3 days before lambing resembled the richer samples in its nitrogen distribution and agglutinin titre. The other, from ewe 22, 12 days

Table 1. *Lambs, dams and treatments*

Kept on the ewe			No colostrum		
Lamb no.	Ewe no.	Breed	Lamb no.	Ewe no.	Breed
512	178	Cheviot	452	255*	Blackface
518	191	Cheviot	415	232	Blackface
672	46	Half-bred	514	190	Cheviot
673	42	Half-bred	674	28	Half-bred
675	28	Half-bred			
678	51	Half-bred			
686	22	Half-bred			

Colostrum delayed				
Lamb no.	Ewe no.	Breed	Delay	Method of receiving colostrum
680	53	Half-bred	12 hr.	Returned to dam
683	13	Half-bred	24 hr.	Returned to dam
682	26	Half-bred	29 hr.	Given 200 ml. colostrum from ewe 53
519	191	Cheviot	48 hr.	Fostered on to ewe 172
677	51	Half-bred	3 days	Fostered on to ewe 37
513	178	Cheviot	9 days	Fostered on to ewe 190

* Not immunized.

Table 2. *Nitrogen distribution and titres of ewes' serum and colostrum*

Ewe no.	Days from lambing	Serum				Colostrum				
		N mg./100 ml.			Titre	N mg./100 ml.				Titre
		Protein	Globulin	Albumin		Protein	Casein	Globulin	Albumin	
13	3 before	—	—	—	—	3767	645	2732	390	7500
	0 after	1251	590	661	500	2635	813	1347	475	> 10000
22	12 before	—	—	—	—	5275	755	4045	475	< 25
	0 after	1095	589	506	500	2706	210	2157	339	1000
26	0 after	1082	568	514	2500	4136	1122	2288	726	2500
28		1036	555	481	500	3390	1390	1565	435	1000
37		1241	725	516	2500	1183	613	300	270	1000
51		1015	573	442	10000	2047	879	838	330	2500
53		1170	670	500	1000	2174	875	1096	203	2500
172		900	430	470	1000	3100	1245	1317	538	2500
178		982	486	496	2500	2980	804	1817	359	> 10000
190		928	442	486	5000	2990	1410	1186	394	> 10000
191		900	430	470	1000	2677	1357	955	365	2500
232	0 after	1046	442	604	5000	2956	1070	1473	413	10000
	3 after	—	—	—	—	1126	734	253	139	2500
	9 after	—	—	—	—	697	588	41	68	250
	36 after	1213	568	645	1000	687	566	56	65	< 25
255	0 after	904	354	550	—	3742	730	2445	567	—
	2 after	—	—	—	—	1191	756	277	158	—
	7 after	—	—	—	—	786	629	66	91	—
	40 after	1242	661	581	—	859	696	63	100	—

before lambing was most concentrated containing 5 g./100 ml. protein nitrogen of which 77 % was globulin nitrogen but its titre was less than 1 in 25. However, the colostrum from the same ewe at lambing had a titre of 1 in 1000. This suggests that the antibodies may be secreted in the colostrum over a relatively short period immediately before lambing. Mason *et al.* (1930) found antitoxin to lamb dysentery present 12 days before lambing, but their ewes had been immunized throughout pregnancy.

The changes in the lambs' serum proteins. The changes shown in Table 3 and Fig. 1 illustrate the effects of the post-natal occurrence and subsequent cessation of colostrum absorption. The degree to which they do so varies with the method of analysis.

The analysis of globulin nitrogen by salt precipitation showed a marked temporary increase in both absolute and relative concentrations after ingestion of colostrum, when this took place at birth or after 12 hr. delay, inconclusive changes when the delay was 24, 29 and 48 hr. and a gradual increase in absolute but not relative concentration over the period of observation when the delay was longer or when no colostrum was given.

The electrophoretic results showed similar trends in protein composition, but in addition an increase in the proportion of globulin when the delay was 24 hr. The approximate average values of the relative globulin concentrations were 21 % at birth, 55 % a few days after absorption of colostrum and 33 % 5 or 6 weeks later. These changes were mainly due to the globulin referred to as component 2. Its corresponding values were 5, 31 and 11 %

respectively. Component 1 remained at about 22 % throughout. By the time the lambs were 5 or 6 weeks old there was little difference in their serum globulin determined by either method whether they had shown absorption or not.

The agglutinin titre clearly showed absorption of globulin in the lambs receiving colostrum up to 29 hr. but not after 48 hr. or more delay. The titres of animals showing absorption rose from negligible values at birth to between 1 in 125 and 1 in 5000 after ingestion of colostrum and fell to quite low values 5 or 6 weeks later.

Comparison of the results obtained on the serum of lambs 512, 518, 678 and 686 (Table 3) with those obtained on the corresponding ewes, 178, 191, 51 and 22 respectively (Table 2) shows that colostrum ingestion and absorption had given to the lamb for the time being a globulin-nitrogen content and an agglutination titre comparable with that of its dam. The lambs serum taken at varying periods after birth is compared with that of the dam at birth only since data on these ewes' serum at corresponding post-natal periods was not obtained. 5 or 6 weeks later the lambs' globulin nitrogen was much less and its titre, as expected, quite small compared with its dam. During this period the lambs' albumin nitrogen rose to a level comparable to that in the dam.

The relative globulin concentration of the lambs' serum has been calculated from the salt precipitation results and included in Table 3. Comparison with the electrophoretically determined globulin shows that they have the same general trends but

Table 3. *Examination of lambs' serum*

Lamb no.	Delay	Age (days)	Nitrogen distribution (mg./100 ml.)			Titre	Percentage composition				
			Protein	Globulin	Albumin		By electrophoresis			By MgSO ₄ pptn. globulin	
							Albumin	Com- ponent 1	Com- ponent 2		
											Globulin
Kept on the ewe											
512	—	0	739	270	469	<25	73.8	22.0	4.2	26.2	37.2
		2	785	421	364	2500	34.6	22.6	42.8	65.4	54.2
		12	1030	492	538	2500	52.3	27.7	20.1	47.7	48.5
		33	965	353	612	500	—	—	13.5	—	37.4
518	—	2	909	476	433	1000	49.0	23.7	27.3	51.0	53.2
		10	936	313	623	250	53.5	28.0	18.5	46.5	34.1
		36	882	248	634	50	63.2	20.1	16.7	36.8	28.8
678	—	0	713	269	444	<2	76.3	21.1	2.6	23.7	38.3
		4	953	456	497	500	50.7	24.9	24.4	49.3	48.7
		10	920	352	568	125	—	—	—	—	39.2
		33	1077	488	589	<25	68.1	25.3	6.6	31.9	46.0
686	—	$\frac{1}{2}$	878	481	397	250	53.9	20.9	25.2	46.1	55.5
		$2\frac{1}{2}$	—	—	—	500	35.6	23.0	38.4	61.4	—
		10	982	445	537	1000	51.6	24.5	23.9	48.4	46.2
		51	935	294	641	<25	—	—	—	—	32.2
Colostrum delayed											
680	12 hr.	0	801	291	510	<2	—	—	—	—	37.0
		3	1029	542	487	2500	40.7	24.5	34.8	59.3	53.5
		10	1042	502	540	250	51.6	25.7	22.7	48.4	49.0
		33	847	315	532	125	74.2	14.6	11.2	25.8	38.0
683	24 hr.	$\frac{1}{2}$	787	316	471	<2	72.8	21.1	6.1	27.2	40.8
		$3\frac{1}{2}$	893	367	526	1000	61.9	27.0	11.1	38.1	41.8
		10	752	278	474	50	62.2	28.0	8.8	36.8	37.7
		29	762	270	492	50	69.1	23.3	7.6	30.9	36.2
682	29 hr.	0	717	289	428	16	—	—	—	—	41.2
		10	860	380	480	1000	—	—	—	—	45.0
		31	812	303	509	125	—	—	—	—	38.0
519	48 hr.	2	597	182	415	<2	60.4	26.7	12.9	39.6	31.2
		5	715	265	450	<25	64.9	—	—	35.1	38.0
		10	759	149	610	<2	65.9	27.4	6.7	34.1	20.0
		36	929	344	585	<2	64.3	23.4	12.3	35.7	37.7
677	3 days	0	677	235	442	<2	—	—	6.2	—	35.3
		3	—	—	—	<2	71.4	23.8	4.8	28.6	—
		5	620	219	401	<2	—	—	—	—	36.2
		10	792	249	543	<2	68.5	23.4	8.1	31.5	32.0
		33	785	283	502	<2	71.7	18.5	9.7	28.2	36.6
513	9 days	0	529	194	335	<25	—	—	—	—	37.2
		9	757	262	495	<25	61.4	34.3	3.9	38.6	35.3
		12	812	228	584	<25	59.2	34.1	6.7	40.8	28.8
		33	792	241	551	<25	58.1	31.7	10.2	41.9	31.2
No colostrum											
452	—	0	731	297	434	—	—	—	—	—	41.4
		2	697	286	411	—	—	—	—	—	41.7
		7	741	309	432	—	—	—	—	—	42.3
		40	898	329	569	—	—	—	—	—	37.2
415	—	0	783	265	518	<25	—	—	—	—	34.5
		3	805	255	550	<25	—	—	—	—	32.4
		10	745	242	503	<25	—	—	—	—	33.2
		36	902	344	558	<25	—	—	—	—	38.8

Fig. 1. Electrophoretic patterns of lambs' sera in phosphate buffer at pH 8.0 and ionic strength 0.2. The lambs were given colostrum after various periods of delay and bled at different ages.

Table 4. Examination of lambs' urine

Lamb no.	Delay	Age (days)	Protein	Globulin	Titre	Lamb no.	Delay	Age (days)	Protein	Globulin	Titre
Kept on the ewe						Colostrum delayed					
672	—	1	+++	++	128	580	12 hr.	1/4	—	—	<2
		2	+	+	16			1 1/4	+++	+	32
		3	tr.*	tr.	8			2 1/4	+	+	8
		4	tr.	tr.	<2			3 1/4	tr.	tr.	<2
673	—	1	++	+	32	683	24 hr.	1/8	—	—	<2
		2	+	tr.	32			1 1/8	+	tr.	16
		3	tr.	tr.	8			2 1/8	+++	+	32
		4	tr.	tr.	2			3 1/8	+	+	32
		10	tr.	tr.	<2			10	tr.	tr.	<2
675	—	1	+++	++	16	682	29 hr.	1/4	+++	+++	—
		2	+++	++	4			1 1/4	+	tr.	16
		3	tr.	tr.	<2			2 1/4	tr.	tr.	8
686	—	1/4	+++	+++	32			3 1/4	tr.	tr.	<2
		1 1/4	+	+	8	519	48 hr.	1	—	—	—
		2 1/4	tr.	tr.	<2			2	tr.	tr.	—
								3	tr.	tr.	—
		No colostrum				677	3 days	1/8	—	—	<2
								4 1/8	—	—	<2
514	—	0	tr.	tr.	—	513	9 days	5	—	—	—
		3	tr.	tr.	—			9	tr.	tr.	—
674	—	1	tr.	tr.	2			10	tr.	tr.	<2
		2	—	—	<2			12	tr.	tr.	<2

* tr. = trace.

are by no means in good or regular agreement. In only eleven out of twenty-eight cases the agreement was within $\pm 10\%$. This may be explained in part by an observation of Jager, Schwartz, Smith, Nicholson & Brown (1950) that too vigorous shaking of serum saturated with magnesium sulphate before filtration may seriously affect the results by protein denaturation.

Results on lambs' urine. These are reported in Table 4 and appear to provide a convenient index of the time of ingestion of colostrum. Proteinuria was marked when ingestion took place at birth and up to 29 hr. after birth; proteinuria was absent or only slight when colostrum was received after 48 hr. or not at all. The proteinuria appeared to include globulin and to be accompanied by an appreciable agglutinin titre. These effects became negligible in a few days.

DISCUSSION

The experimental data suggest that the changes observed in the lamb serum indicate the absorption of immune globulin from colostrum. These changes, together with those in the urine, show that ingestion of colostrum by the lambs up to some time between 29 and 48 hr. after birth leads to the absorption of immune globulin and the appearance or increase of a slow-moving electrophoretic component referred to as component 2. This finding may be compared with that obtained by Hansen & Phillips (1947) on the calf. Their electrophoretic studies showed absorption of gamma globulin at 24 but not at 42 hr. after birth.

The urine tests showed that the kidney of the newborn lamb appears to possess an unusual permeability, which persists for at least the same period

after birth as that observed in the alimentary canal.

The permeability or otherwise of the different types of placentae has usually been explained in terms of the number of layers of tissue concerned. The present results indicate that this may not be so important as the properties of a particular layer since the impermeability of the placenta is in marked contrast to the permeability of the numerous tissue layers of the udder, alimentary canal and kidney. The results also show that the placental impermeability is not absolute, as sometimes a small amount of component 2 and a discernible agglutinin reaction could be obtained in the lambs' serum at birth (Table 3).

SUMMARY

1. Immunological, salt-precipitation and electrophoretic methods of investigation have been combined in a study of (a) the changes in serum proteins of lambs on ingestion of colostrum at birth, (b) the effect of delaying the ingestion of colostrum on these changes and (c) the occurrence of any proteinuria in this connexion.

2. The results show that there is an increase in serum globulin largely due to one electrophoretic component and an appearance of antibody, when colostrum is given up to 29 hr. but not at 48 hr. or more after birth.

3. Following the ingestion of colostrum the lambs showed a marked proteinuria, characterized by the presence of globulin and antibody.

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