EVIDENCE FOR EPISTATIC ACTION OF GENES FOR ANTIGENIC SUBSTANCES IN SHEEP¹

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PREVIOUS studies on the blood of sheep (ANDERSEN 1938; YCAS 1949) have shown the presence on the erythrocytes of an antigenic character, called R, demonstrable by a normally occurring antibody present in the sera of some sheep lacking the R substance. The report by YCAS suggested that the R character was inherited as a dominant in contrast to its absence. Further investigations by STORMONT (1951) showed that sheep bloods, which failed to react with anti-R reagents (i.e., typing fluids specific for the R-substance), might react with normal sera of certain cattle which had been absorbed with sheep cells of group R. This reagent obtained from cattle serum was called anti-O. In tests of over 100 sheep bloods with both anti-R and this new reagent (anti-O), the reactions were mutually exclusive. Since the new reagent was not reactive with the cells of heterozygotes, STORMONT suggested that the genes determining the R and O characters were allelic, the gene for R being dominant. However, in this paper the O character will be called "r" in order to have the terminology in concordance with genetical practice.

This report describes a new group "i" of sheep erythrocytes. Cells are classified as belonging to group i if they fail to react with both anti-R and anti-r sera. Further, this report will deal with the serological characteristics and the inheritance of the characters R, r and i.

MATERIALS AND METHODS

The study has been performed on about 400 individual sheep belonging to the University of Wisconsin, of the following breeds: Hampshire (111), Southdown (67), Shropshire (91) and mixed breeds (130).

Blood was taken from the jugular vein into a clean glass tube containing isotonic citrate solution (2 percent sodium citrate and 0.5 percent sodium-chloride), when the sample was used for a cell suspension. If serum was desired, citrate was not used, and the serum was permitted to clot. After centrifugation the serum was drawn off and stored. The sera were not heated to destroy complement, since it was observed that unheated sera did not contain sufficient complement activity to interfere with the results.

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Sera from different R negative and r positive (group r) individuals were used as testing fluids (reagents) for the R character. DR. C. STORMONT, of the School of Veterinary Medicine, Davis, California, kindly furnished normal serum from one cow, from which anti-r reagents could be produced by absorption with R cells. In general, absorptions performed in the course of these investigations were carried out as follows: 1 volume of appropriately diluted serum was added to 1 volume of washed, packed erythrocytes. The mixture was shaken and allowed to incubate at room temperature ($24^{\circ} \pm 2^{\circ}\text{C}$) for one-half hour. The tubes were centrifuged, the supernatant serum removed and placed in a tube containing an equal volume of freshly washed erythrocytes, shaken and then allowed to incubate for 30 minutes at room temperature. After another half hour in the refrigerator ($2^{\circ} \pm 2^{\circ}$), the tubes were centrifuged and the serum was collected.

The technique involved in the lytic test has been described elsewhere (STORMONT and CUMLEY 1943; YCAS 1949). Briefly, 0.1 ml of diluted anti-serum and 0.05 ml of a 2.2–2.5 percent cell suspension were mixed in a test tube. After 15 minutes incubation at room temperature, 0.05 ml of complement, diluted 1:2, was added. Rabbit serum, which was used as complement, usually contained species antibodies for sheep cells. Hence, it was absorbed with pooled sheep cells before it was used as complement. (The absorption for complement was performed by mixing equal volumes of fresh, undiluted rabbit serum and packed, washed sheep cells for ten minutes in a tube surrounded by crushed ice.) The tubes containing antiserum, cells and complement were shaken and allowed to incubate at room temperature for one-half hour, after which they were again shaken. The test was read after an additional one and one-half to two hours incubation and checked after still another one and one-half hours incubation.

The different antisera were ordinarily used in a dilution of 1:4, which was more than sufficient to give complete lysis, if the antigenic factor was present on the test cells. However, the cells of young lambs were tested at a serum dilution of 1:2. All cells which failed to react with either anti-R or anti-r sera were re-tested at a dilution of 1:2 of both antisera.

Inhibition tests were performed to estimate the occurrence and the relative concentration of soluble antigenic substances in the sera of different individuals. If a serum contains serologically active substances identical with or related to substances on the cells, then the homologous antiserum, if mixed with sera containing the soluble substance, will lose its ability to lyse the homologous cells. The serum whose inhibiting activity is to be measured is called the "inhibitant." This is diluted in doubling dilutions. The highest dilution inhibiting the reaction between the antiserum and the test cells will give an approximate measure of the amount of the soluble substance present in the serum. The inhibition test was performed as follows: 0.05 ml of the inhibitant and 0.1 ml of the antiserum (reagent) were mixed in a test tube and permitted to stand for one-half hour, after which the appropriate blood cells (which usually were lysed completely by the reagent) and the comple-

ment were added in that order. Controls were included to test for antibodies in the inhibitant against the test cells.

STORMONT (1949) and STONE (1953) have reported that it was possible to convert erythrocytes of cattle from J negative into J positive by treating them with serum from J positive individuals which contained the soluble, serologically active J substance. In order to determine if it was possible to "coat" sheep erythrocytes of one type with the soluble substance of another type, "coating experiments" were performed. The cells of one group were mixed with serum of another group in the proportion 1:14 and allowed to stand in a water bath at 37°C for 5 hours, after which they were placed in a refrigerator overnight. If the cells were not coated after that time, they were removed to fresh serum and incubated again for 5 hours at 37°C. When in the water bath the tubes were shaken once each hour.

EXPERIMENTAL RESULTS

Previous studies by YCAS (1949) and STORMONT (1951), and the present studies indicate that sheep bloods may be classified into groups as outlined below:

R-substance	r-substance	Anti-R	Terminology of STORMONT	Proposed terminology
+	0	0	R	R
0	+	+	O	r
0	0	+ or 0		i

R indicates the presence of the R substance on the cells and in the serum; r indicates the presence of the r substance on the cells and in the serum, which also contains anti-R; i refers to a group lacking R and r substances, and anti-R is present in the serum of only some of the individuals. The i group was not detected prior to this report.

Of the 399 bloods tested, 222 were classified as R, 151 as r, and 26 as i (16 adults and 10 lambs) since their cells reacted neither with anti-R nor with anti-r sera. Since lambs normally develop the R or the r substance on their cells before they reach an age of 50 days, and those classified as i ranged in age from 80–129 days, it seems very unlikely that the absence of both R and r on the cells of these 26 animals is due to a delayed appearance of either R or r.

YCAS (1949) demonstrated by inhibition tests that sera from individuals classified as group R contained a soluble substance, which inhibited the reactions between anti-R sera and R cells. Similar results were observed by NIEMANN SORENSSEN, RENDEL and STONE (1954) for the sera of group r, since such sera inhibited the reactions between the r-reagents and r cells. These observations indicated that sera of group r contained a soluble substance identical or closely related to the antigenic substance of the r cells.

In this study 20 r individuals were tested for the presence of R antibodies in their sera. Thirteen sera were found to contain strong anti-R, while 7 had

only weak antibodies, which presumably were anti-R. This conclusion is based on the fact that these sera did not lyse any cells of group r, but reacted with R cells which gave strong lysis with other anti-R sera, and failed to react with cells giving weaker reactions with these strong anti-R sera. However, the possibility that these reactions were due to other normal antibodies cannot be ruled out entirely.

The sera of 14 adults and 4 lambs of group i have been tested for substances inhibiting anti-R and anti-r sera. For each of these antisera not only i serum, but also, as controls, R and r sera were used as inhibitors. At first the tests were difficult to interpret, since it was found that the i serum consistently inhibited the anti-R activity of r serum one or two dilutions more than did the control r sera, while i sera and R sera inhibited anti-r approximately equally. Consequently the inhibiting activity of i and R serum on anti-r serum should be considered as non-specific. The fact that i serum inhibited anti-R sera more strongly than did the r serum could be interpreted as meaning that the sera of individuals classified as i may contain a small amount of the R substance, in a concentration too low to be acquired by the cells but great enough to cause observable inhibition of anti-R sera. This finding is similar to certain observations by STONE (1953) in studies on the J-substance of cattle. However the results might also be interpreted as nonspecific inhibition, since r sera used in the inhibition test with anti-R had previously been absorbed with R cells to remove anti-R and still showed a slight inhibition of the reaction between R cells and R-antisera. This difference in inhibiting capacity between the r (anti-R) and i sera might be accounted for by assuming that the absorption of the r sera by R cells decreased their normal and possibly non-specific inhibiting capacity.

To investigate the question further, five sera from i individuals, three from R and three from r, were absorbed by pooled cells of group R and r. Cells of type r were included to absorb the reactions from other normal antibodies, which might occur in any of these sera. The same types of cells were used for absorption and as test cells. Two anti-R sera were included. One of these anti-sera was used at dilutions of 1/256 and 1/128 (containing 4 and 8 units of hemolysin, respectively,) the other was used at dilutions of 1/128 and 1/32 (containing 4 and 16 units of hemolysin). (The highest dilution of a reagent giving definite hemolysis is taken to contain one unit of hemolysin.) The anti-r serum had 8 and 4 units of hemolysin in its different dilutions. In table 1, the results of the inhibition tests are shown for one representative serum from each of the three groups of inhabitants. It may be seen that the serum from an individual of group i does not inhibit the R-antibodies more than the non-specific inhibition of the r-serum. Also there was no significant difference between i and R sera in their inhibiting action of anti-r sera.

In addition, cells from 10 more sheep of group-i have been used in absorptions of anti-R and anti-r sera. The specific antibodies in the respective antisera were not removed. Thus the conclusion seems justified that sheep of group i do not contain either R or r substances on their cells or in their sera.

The question arises as to whether the sera of group i contain R- or r-antibodies. The sera of 23 individuals of group i (14 adults and 9 lambs with an average age of 117 days) have been tested for R- and r-antibodies. None of these sera were found to have anti-r, neither did any of the i sera from adults contain R-antibodies. Two of the sera from lambs showed strong or intermediate R-antibodies, while 7 lacked anti-R. One of these latter had strong normal antibodies, which did not parallel either anti-R or anti-r.

The marked variation in the antibody titer of anti-R normal sera reported by YCAS (1949) suggests the possibility that some individuals of group i, which at a certain time lack R-antibodies might at another time contain them. However, nine of the adult individuals of group i have been tested in two succeeding months and two individuals have been tested five times over a period

TABLE 1

The inhibition of anti-R and anti-r sera with sera of the groups, R, r and i.

Inhibitant		R-serum				r-serum				i-serum			
Reagent		Anti-R		Anti-r		Anti-R		Anti-r		Anti-R		Anti-r	
Units of hemolysin		8	4	8	4	8	4	8	4	8	4	8	4
Dilution of inhabitant	1/1	0	0	4	0	4	1	0	0	4	tr	4	3
	1/2	0	0	4	3+	4	3	0	0	4	3	4	4
	1/4	0	0	4	4	4	3+	0	0	4	4	4	4
	1/8	0	0	4	4	4	4	0	0	4	4	4	4
	1/16	0	0	4	4	4	4	0	0	4	4	4	4
	1/32	3	0	4	4	4	4	4	0	4	4	4	4
	1/64	4	0	4	4	4	4	4	0	4	4	4	4
	1/128	4	1	4	4	4	4	4	0	4	4	4	4
	1/256	4	2	4	4	4	4	4	0	4	4	4	4
Control (no inhibitant)		4	4	4	4	4	4	4	4	4	4	4	4

Symbols: 4 = complete hemolysis; 3 = hemolysis not quite complete; 2 and 1 = incomplete or intermediate hemolysis; Tr = weak hemolysis; 0 = no visible hemolysis.

of 11 months (once in June 1952 and in February, March, April and May, 1953) and no antibodies against either R or r cells have been found. Thus it seems justified to conclude that some individuals of group i lack R-antibodies and that presumably all individuals of group i lack r-antibodies. A tentative genetical interpretation of the findings that some individuals of group i have anti-R, while others lack it, will be discussed later.

YCAS (1949) found that the R substance was present at birth in the serum but not on the cells of R lambs. The substance was presumably acquired by the cells from the serum, at an average age of three weeks. The results of the present study agree with those of YCAS on the R character and show in addition that the r substance was not present on the erythrocytes of newborn lambs.

Among the cells of a group of 78 lambs of an age varying from 9 to 34 days, 8 were of group R and 16 of group r, while the remaining 54 were completely negative or showed only weak reactions with a specific reagent. (A

reaction was designated as positive only if half or more of the cells were lysed.) The youngest age at which the lambs could be shown to belong to group R or r was 19 and 20 days, respectively. It was found that the majority of the 54 "double negatives" developed the R and r factors on their cells at an age of about one month. Furthermore, inhibition tests with the sera of four lambs showed that the r substance was present in the serum of lambs of group r before it was detectable on the cells.

It can be concluded that there are not two, as previously believed, but three distinct types of sheep blood: (1) R, characterized by the R-factor on the cells and the soluble R substance in the serum; (2) r, having the r factor on the cells and the soluble r substance and R-antibodies of varying strength in the serum; (3) i, having neither R nor r on the cells or in the serum. Some individuals of this group have demonstrable R-antibodies in their sera.

Coating experiments

To investigate if it was possible for the erythrocytes of sheep of one type to acquire the soluble substance of another type, coating experiments were performed by the method described earlier. When cells of groups r and i were treated with sera from individuals of group R a significant coating was observed, after the cells had been treated 5 hours at 37°C and 15 hours in the refrigerator and then tested with a reagent for R. After repeated incubation of the cells in fresh serum the strength of the subsequent lytic reactions increased and in some cases complete lysis was observed. Thus it was possible to change r cells into those reacting with both anti-R and anti-r sera, and to convert i cells into R-cells. The r cells coated with R substance behaved as expected of heterozygotes, such as the AB or MN of man, or the F^FF^V of cattle, in that they were reactive with the reagents for both contrasting antigenic characters. However, STORMONT (1951) has reported, and our findings are in agreement, that the cells of heterozygotes (*R/r*) are not reactive with the reagent for r.

Some variability was observed in the ability of different sera to coat the cells of an individual, and also in the ability of different cells to be coated. (Comparable variability was also noted by STONE (1953) in experiments on J in cattle.) When r sera were used for coating of i cells only insignificant coating was observed after the first treatment (5 hours at 37°C and 15 hours in refrigerator). After prolonged treatment, however, it was possible to convert i cells into cells of group r. Thus it should be theoretically possible to change cells of group i into cells reacting with both anti-R and anti-r sera, if first treated with r and *later* with R sera, because r sera seem invariably to contain antibodies against R. On the other hand, it has not been possible to coat R cells with r substance, because we have never been able to exhaust the r sera of R-antibodies and R cells were therefore lysed during the coating treatment. However, these results indicate that both the R or r soluble substances, present in sheep serum of a particular group, are acquired by the erythrocytes from the surrounding serum.

Genetical studies

This study includes 148 matings, divided into 9 sire-families. Five sires were of group R, three of group r, and one of group i. The numbers and blood types of the offspring from the different matings are summarized in table 2. The matings of $R \times R$ individuals gave 30 offspring with R, 5 with r, and 2 with i. From $r \times r$ matings only r progeny (44) were obtained, and in matings $R \times r$ and $r \times R$ there was a slight excess of R in comparison with r individuals (55:35). These results would be expected, if R is dominant to r. Thus these data agree with the proposal of STORMONT (1951), that the gene determining the group R is dominant over that determining the r substance.

From the data summarized in table 2, it may be seen that offspring of type i appeared in the offspring of matings of $R \times R$ and $R \times r$. And an exception to the usual rule within all species that an individual carries a cellular antigen

TABLE 2
The numbers and classes of offspring from different matings.

Blood type of parents		No. of sires	No. of matings	Offspring with blood type			Total No. of offspring
sire	dam			R	r	i	
R	R	5	29	30	5	2	37
R	r	5	12	12	1	2	15
r	R	3	52	43	34	0	77
R	i	1	1	1	0	0	1
i	R	1	11	7	2	8	17
r	r	3	28	0	44	0	44
r	i ¹	2	6	7	1	0	8
i	r	1	7	4 ²	3	2	9
i	i	1	2	0	0	2	2
Total			148	104	90	16	210

¹All parents and offspring in this kind of mating have been tested at least twice, some three or four times, with anti-R (sheep) and anti-r (cattle) sera.

²Two of these with R were adults.

only if either or both parents also possess it may be noted in that progeny of group R appeared from the reciprocal matings of $r \times i$. Hence it appears that the i group behaves in inheritance as a recessive, but definitely not as a recessive to either R or r. For further critical analysis, the blood types of the individuals involved in pertinent matings of these three rams are therefore given in table 3. It may be deduced from table 3 that ram 468 is R/r , on the assumption that R is dominant to r, since from two matings (1 and 5) to ewes with R, both R (B41) and r (B40, 228) offspring were obtained. However, two offspring of group i (252, 287) were sired by ram 468, one from a mating (8) to a ewe of group r, the other from a mating (11) with a ewe of group R. Parallel results are shown in the three matings (86, 88, 89) given for ram 876 (group R) in that this ram sired both R and r lambs from mating to a ewe with r, an r lamb from a ewe with R and an i lamb from a ewe with R. Further, the two offspring with i (numbers 252 and 287), from sire 468,

in matings (17, 19) to a ram with *r* (158987) produced a lamb with *R* (mating 17) and lambs with *R* and *r*, respectively. The ewe 252 with blood type *i* must have carried the gene for *R* which was expressed in her offspring B5. The data of table 2 are also pertinent in which the ram of blood type *i*, in matings to ewes with *R*, produced 7 offspring with *R*, 2 with *r*, and 8 with *i*. The data in tables 2 and 3 are consistent with the assumption that the genes *R* and *r* produce their respective products only when the dominant allele of *i* at another locus is present.

Additional evidence of the inheritance of the groups *R*, *r* and *i* is given in the pedigrees of figures 1 and 2, in which the phenotypes and probable genotypes are pictured for individuals representing three generations. From the data of table 3, it appears that the genotype of ram 468 is *I/i R/r*, that of ewe

TABLE 3
Selected data from three sire families.

Mating number	Sire		Dam		Offspring	
	No.	Bloodgroup	No.	Bloodgroup	No.	Bloodgroup
1	468	R	257	R	B40	<i>r</i>
					B41	R
5	468	R	338	R	228	<i>r</i>
8	468	R	416	<i>r</i>	252	<i>i</i>
9	468	R	416	<i>r</i>	333	R
11	468	R	3665	R	287	<i>i</i>
17	158987	<i>r</i>	252	<i>i</i>	B5	R
19	158987	<i>r</i>	287	<i>i</i>	B25	<i>r</i>
					B26	R
86	876	R	608	<i>r</i>	B656	<i>r</i>
					B657	R
88	876	R	808	R	B662	<i>i</i>
89	876	R	826	R	B655	<i>r</i>

The cells of all individuals in table 3, except from numbers 257, 333, B40 and B41 have been tested at least twice. The prefix B indicates that the individuals were born in 1953; other sheep are one year of age or more.

416 is *I/i r/r*, that of both 252 and 287 is *i/i R/r*. It is also apparent from the pedigree in figure 1 that ram Moore has the genotype *i/i R/r*, because matings with *r* individuals resulted in offspring of the *R*, *r* and *i* groups. Furthermore Nos. 332 and 333 must have the genotype *R/r* and the latter must be heterozygous *I/i*, because her lambs B23 and B24 from a mating to Moore were of group *i*. The origin of one of the genes for *i* in each of these twins can be traced back to one of their maternal grandparents. The other gene for *i* came from the sire (Moore). Similarly the *R* gene in B5, which was not expressed in its mother (252) because of the action of the homozygous *i/i*, was derived from its maternal grandfather. As shown in figure 2, the *R* gene in B26 can in a similar fashion be traced back to one of its maternal grandparents. When the ram of group *i* (Moore) was mated to two ewes of group *i* (as in figure 2), the offspring were of group *i* as expected. The breeding records of the flock showed that ewe 287 of figure 2 had been mated

to the ram Moore, and later to ram 158987. If the progeny No. 380 were sired by Moore, the gestation period was 150 days as compared with 168 days if 158987 were the sire. The gestation period in sheep is 140 to 160 days (DUKES 1947). Thus it seems somewhat more probable that the ram Moore is the sire of lamb 380, and this is indicated by the dotted line in figure 2 between

FIGURE 1.—Pedigree showing inheritance of R, r and i groups in sheep.

FIGURE 2.—Pedigree showing inheritance of R, r and i groups in sheep.

Moore and 287. The gene for i is undoubtedly not sex-linked, because ram 468 ($I/i R/r$) has two daughters (252 and 287) of group i.

The data of the present study are not well suited for studies of the gene frequencies, because they are derived from four small populations within each of which only a few rams have been used. However, it may be possible to get a rough estimate of the gene frequencies. Only ewes were used in the calculations because the lambs formed a small number of half-sib-groups. The

degree of relationship among the ewes has been disregarded. Each ewe has only been counted once, even if it has been used in more than one mating.

The numbers of ewes of the different breeds and their respective blood-groups are summarized in table 4. A χ^2 -analysis was performed to test if the four subpopulations could be treated as one population in the calculations of the gene frequencies. The numbers of individuals of group *i* were very small. However, the ewes of group *i* in the Hampshire and Shropshire breeds have all been shown to be of the genotype *i/i*, *R/-*. Thus they were counted as *R* individuals. On the other hand the four ewes of group *i* in the Southdown and "Mixed" breeds have been omitted, because they could be any one of the genotypes *R/R*, *R/r* or *r/r*. The χ^2 -test indicates that the data could be

TABLE 4
The blood groups of ewes of four different classes of sheep.

Breed	Number of ewes of group			Total number
	I		i	
	R	r		
Shropshire	15	10	2 ¹	27
Hampshire	26	15	4 ¹	45
Southdown	15	3	2	20
Mixed	37	16	2	55
Total	93	44	10	147

¹These ewes have been proven to be of the genotype *R/-*, *i/i*.

pooled ($\chi^2 = 2.51$, D.F. = 3). The pooled gene frequencies were as follows: *R* = 0.45, *r* = 0.55, *I* = 0.74 and *i* = 0.26. The calculations have been made under the assumption that individuals of the genotype *i/i r/r* show the phenotype *i*. This question will be discussed below.

DISCUSSION

KACZKOWSKI (1928) and YCAS (1949) classified sheep bloods according to their reactions with the normally occurring anti-*R*. Thus they did not have the test fluid, or reagent, to distinguish between individuals of group *r* and *i*, neither of which react with anti-*R*. However, both authors subdivided these non-reacting animals (*r* and *i* in this paper) into two groups according to the presence or absence of anti-*R* in the serum. It might be expected that some individuals of group *R* would have arisen in the different matings between non-reactors reported by KACZKOWSKI and by YCAS. Actually, YCAS observed one individual of group *R* from such a mating. She suggested that the *R* lamb in this case was due to an error in the breeding records. However, such a result would be expected in the light of the present findings.

One possible explanation of the action of the gene for *i* might be that it prevents the secretion of the *R* and presumably the *r*-substance into the serum. This is somewhat analogous to the proposed action of the secretor gene (*S*)

in humans described by SHIFF and SASAKI (1932). Individuals which carry homozygous recessive gene (*ss*) do not have the A, B or O substances in their body fluids and secretions, such as saliva and tears, whereas these are found in all individuals carrying the normal allele (*S*), with the particular substance of the secretions dependent upon the genotype for A, B or O of the cells. Further, the antigens A, B, and O of the red blood cells of man are not known to be affected by the secretor (*S*) or nonsecretor (*s*) genes. In contrast, in the blood of sheep the presence of the R or r substances on the cells appears to be entirely dependent upon its prior appearance in the plasma. The genes for the R and r substances of sheep then, are capable of functioning, at least in the plasma and blood cells, only in the presence of the allele (*I*) to the recessive suppressor gene (*i*). By analogy with other genetic phenomena, it might be proposed that the gene *i* when homozygous in some way inhibits or suppresses an enzyme which is active in sheep with gene *I*. If this is correct, such an enzyme should be sought in an organ, or organs, as the liver, concerned with the synthesis of serum and serum constituents. Other explanations cannot be excluded, but this relatively simple one seems reasonable.

Eight of the 23 individuals of group *i* included in the present studies have been shown to be of the genotype *i/i R/-* because when mated to *r/r* they have produced offspring with R. The remaining 15 may be of any of the genotypes *R/R*, *R/r* or *r/r*. However, it cannot be established that any of these are of the genotype *i/i r/r*. Such an individual must result from a mating *r* × *r*. That no individuals of group *i* have occurred among the 44 offspring produced in the matings of *r* × *r* may be explained (a) by assuming that the three rams of group *r* are homozygous *I/I*, or (b) that the *i* gene in homozygous condition suppresses only the formation of the R-substance, not the r-substance. Only one of the three rams had sufficient offspring to conclude with reasonable certainty that it was *I/I*, while the other two could have been either *I/I* or *I/i*.

The R, r and *i* classes of blood in sheep have considerable resemblance to the Lewis blood groups in man, according to the explanation of the inheritance of the Lewis groups by ANDRESEN and JORDAL (1949). A recent proposal of the inheritance for the Lewis system has been advanced by GRUBB (1951) which, if applied to the R, r and *i* groups in sheep, would mean that the R and r substances are determined by a complicated interaction of genes at different loci, as R and R' at one locus, r and r' at another, the phenotype *i* being present if the genotype is *R'/R' r'/r'*. The most pertinent argument against such an explanation for sheep bloods is the appearance of R, r and *i* offspring from *r* × *i* matings, as was actually observed. For this and other reasons, it seems much more reasonable to explain the situation by assuming that the R and r substances are produced by alleles, and that a recessive gene *i*, at another locus, when homozygous suppresses the expression of the R substance, and presumably also the r substance.

Another interesting aspect of the R, r and *i* groups of sheep blood is the observation that all sera from individuals of group *r* have contained R-anti-

bodies, while only two out of 23 individuals of group i showed anti-R. BERNSTEIN (cited by WIENER 1943) postulated that the antithetical relationship in humans between α -agglutinin and antigen A, and between β -agglutinin and antigen B, could arise under the assumption that all humans produced α - and β -agglutinin, but that α -agglutinin is absorbed if antigen A is present on the cells, as is β if the cells possess β . If such a system were operating in sheep to determine the presence of R-antibodies, one would expect all sheep of the groups r and i to contain anti-R. Since only two of the twenty-three individuals of group i possessed anti-R, it appears highly probable that the explanation proposed for the normal antibodies of humans is not valid for sheep. Further, there remains the possibility that the gene for the r substance has a pleiotropic effect, to produce the r substance in the plasma in the presence of the *I* gene, and anti-R in the serum. Under this proposal, which parallels a similar explanation for the reciprocal appearance of the iso-agglutinins and the A and B antigens of humans (COTTERMAN 1952, among others) group i animals of the genotype *i/i R/-* should not, while those of the genotype *i/i r/r* should, contain anti-R in their sera. This possibility can be substantiated or disproved only after the sera of sheep of the genotype *i/i r/r* can be tested.

On the assumption that the explanation proposed above of the inheritance of the R, r and i classes of the bloods of sheep is correct, it provides an example of a recessive gene (*i*), epistatic to genes (*R* and *r*) which effect antigenic substances of the plasma and secondarily of the blood cells. If the epistatic action of the *i* gene had its primary action on the cells, it would represent a different effect from any known at present of genes which produce cellular antigens. In the light of the present evidence, the authors favor the explanation that the *I* and *i* genes act on the secretion of R and r substances into the serum, from which they are taken up by the blood cells.

SUMMARY

The present report describes three classes of the blood of sheep. (1) R blood, characterized by the R-substance on the cells and the soluble R substance in the serum. The R factor is detected by normally occurring antibodies in some sheep lacking R. (2) r blood, having the r-substance on the cells and the soluble r substance and R-antibodies of varying strength in the serum. Some cattle normal sera contain specific antibodies against the r-factor. (3) i blood, having neither R nor r substances in the serum or on the cells. Only some individuals of this group have R-antibodies in their sera.

The R and r substances are not detectable on the cells of lambs until they reach an age of about one month. The respective substances in soluble form are present in the sera before they can be detected on the cells.

It has been possible *in vitro* to change the cells of one group into a second group, by incubating them in serum of the second group. It has thus been possible to produce an artificial cell-type reacting with both anti-R and anti-r sera, by incubating r cells in R serum. These experiments and the gradual development of the R and r substance on the cells of lambs indicate strongly

that the R and r substances are taken up by the cells from the surrounding plasma.

The data on the inheritance of the three classes of blood, R, r and i, are in accord with the assumption that it is controlled by genes at two loci, with the recessive gene *i* epistatic to dominant *R* and probably also to *r*, so that group R is *I/- R/-*, r is *I/- r/r*, and i is *i/i R/-* (and probably *i/i r/r*).

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