Genet. Res., Camb. (1968), **11**, *pp.* 271–287
With 4 text-figures
Printed in Great Britain

The genetic control of antibody production. A study of isoimmune antibodies in cattle twins*

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(*Received* 15 *August* 1967)

1. INTRODUCTION

The antibody response varies with a number of factors such as the nature and dosage of the antigen administered; the number, frequency and route of injections. The individual differences which remain when these variables are kept equal depend on the previous immunological experience of the recipients and their genetic endowment. There is now substantial evidence that the individual variation in antibody production is at least partly genetic. By short-term selection Fjord-Scheibel (1943) was able to divide a population of guinea-pigs into one line with a very high frequency of diphtheria antitoxin producers and another line where this frequency was very low. Carlinfanti (1948) studied the titres of the isoagglutinins, anti-A and anti-B, in man and found a parent–child correlation for each of these antibodies close to 0·5, indicating a strong genetic control of the antibody titres. Differences between strains of mice in their ability to produce antibodies against natural or synthetic antigens have been reported by a number of investigators, e.g. Davidson & Stern (1954) and Fink & Quinn (1953). McDevitt & Sela (1965), who studied two strains of mice, found marked strain differences in the antibody response against a synthetic multi-chain polypeptide, while the strains responded equally to bovine serum albumin. That the antibody response to complex antigen mixtures may be under less rigid genetic control than the response to more specific entities was shown also by Sang & Sobey (1954) and Sobey & Adams (1961). They estimated the heritability of antibody response in rabbits against an antigenic specificity of the tobacco mosaic virus to be 0·87, while the response against bovine serume albumin showed practically no heritability.

Most studies on the genetic influence of antibody response have been based on hetero-immunizations and substances with complex antigenic make-up. The occurrence of genetically determined red cell antigens (blood groups) in a number of species makes possible studies of the heritability of antibody response against substances occurring normally within the respective recipient species. In the present paper results will be presented of a study on the antibody response of monozygous and dizygous cattle twins against three red cell antigens controlled by three independent genetic loci. Even though the exact chemical nature of these antigens is unknown, their Mendelian inheritance shows that they have well

* The study was supported by a grant from the Swedish Agricultural Research Council (Jordbrukets Forsinkingsråd).

defined and stable characteristics. The aim of the study has been to investigate (*a*) the difference in antigenicity between these three antigens when injected into the same animals, (*b*) the genetic influence on the individual variation in antibody response to a given antigen and (*c*) the correlation between the antibody titres of different populations of antibodies produced in the same animals.

2. MATERIAL AND METHODS

The recipients were selected on the basis of their red cell antigen types from the herd of monozygous (MZ) cattle twins at our department and from a herd of dizygous (DZ) cattle twins at the department of animal feeding and management. All animals were tested with forty-two typing blood reagents detecting antigenic factors controlled by nine different loci: A, B, C, F–V, J, L, M, S–U and Z (cf. Stormont, 1962). An animal with a rare blood type was selected as blood donor. It had the W, F, V, J, M and U′ factors only. Most of the recipients lacked the V, M and U′ factors, and could therefore be expected to produce antibodies against these antigens if inoculated with red cells of an appropriate type. The J antigen, which is detected by naturally occurring isoimmune antibodies, does not normally evoke antibody production when injected into recipients lacking J. The differences if any between the recipients and the donor with regard to the J antigen can therefore be neglected here. Most of the recipients had the F and W antigenic factors.

The diagnosis of zygosity was based on morphological examinations (cf. Brännäng & Rendel, 1958) and tests for red cell antigens and serum protein types (Rendel, 1963). The diagnosis of one heifer pair (205–206) needs special mention. It was originally classified as monozygous and was also included as such in the study. However, before the experiment was completed the members of the pair showed distinct differences in body proportions and colour shade, and research officers in charge of the experimental farm reclassified the pair as probably dizygous. Due to the uncertainty of the diagnosis, pair 205–206 will not be included among either the MZ or the DZ class. Instead some results will be given separately in the discussion.

An admixture of red blood cells (erythrocyte mosaicism) was found in five of the ten DZ pairs; in four of the other pairs the co-twins differed with regard to transferrins or post-albumins, and in the remaining set the twins were morphologically so different that they were classified as definitely dizygous in spite of the fact that they were indentical with regard to the diagnostic blood characters. No single pair showed differences in the ordinary red cell antigens, and all the pairs might therefore very well have had foetal vascular anastomosis, though red cell admixture was proved in some only.

The twin pairs were 12 months of age or older at the onset of the experiment. Pairs of both sexes occurred. To our knowledge, no twin had previously been injected with red cells or any other substance with blood group activity. Most of the pairs had previously been in, or still took part in, some feeding or managerial trials. The environmental conditions of the pairs are summarized in Table 1. The

differences in management between co-twins concerned the age at first calving or the methods of milking. The occurrence of feed-quality differences means that one twin got more hay than the co-twin, while differences in feeding intensity mean that the amount of feed given up to 2 years of age differed between the members. The environmental variation between co-twins was on the average much larger than that usually occurring between members of the same herd.

Table 1. *Summary of the environmental conditions of the twin pairs*

	Number of pairs	Age (months)		No. of pairs with systematic intra-pair differences in:			No systematic intra-pair differences
		Range	Av.	Feeding intensity	Quality of feed	Management	
1. MZ pairs							
Bulls	7	16–23	21·0	0	5	2	0
Heifers	8	12–22	18·6	5	0	3	0
Cows	10	34–93	63·3	0	0	10	0
2. DZ							
Steers	8	13–15	14·3	8	0	0	0
Heifers	1	17	.	0	0	1	0
Cow	1	60	.	0	0	0	1
3. 205–206	.	15	.	.	.	.	1

Two series of immunizations were carried out. The first (on fourteen MZ pairs) was started in July 1963 and the second (on eleven MZ and ten DZ pairs) in February 1965. All the recipients were inoculated 5 times intramuscularly at weekly intervals with 10 ml of blood from the donor. Blood was collected from each individual before the first injection and thereafter 8 times on every 3rd and alternatively 4th day, thereafter twice with weekly and twice with fortnightly intervals. The complement activity of the serum was destroyed by heating to 56° C for 30 min and the samples were thereafter stored at −20 °C.

The specificities of the antisera and their titres were determined in the standard haemolytic test described by Stormont & Cumley (1943) with minor modifications. Each antiserum was tested in serial doubling dilutions against red cells from twenty to thirty individuals of known type, including the donor. The test cells were chosen so that all possible combinations of the antigenic factors of the donor were represented (cf. Table 6). All the 844 serum samples were titred and eighty sera representing fifteen MZ pairs were retested against nine cell samples included in the first titration. The correlation between the titre values on the two occasions was 0·99, thus indicating a good repeatibility of the test. Whenever there was any doubt of the specificity of an antiserum, check absorptions were performed.

The two antisera collected from a twin pair at a given date were always tested simultaneously. The haemolytic reactions were recorded as follows: 0 = no haemolysis, 4 = complete haemolysis; 0+, trace, (tr) 1, 2 or 3, indicating intermediate reactions of increasing strength. The highest dilution of an antiserum which gave a definite positive reaction (trace or higher) was recorded as the titre.

In each of the two series into which the study was divided the titre values were based on the same cells and these carried one of the respective antigenic factors only, i.e. M, V or U′. The titre values were transformed into a log scale where 1 indicated haemolysis at dilution $\frac{1}{2}$, 2 at $\frac{1}{4}$, 3 = $\frac{1}{8}$, etc. To describe the antibody populations and the antibody-producing capacity of the twins, the following data were recorded for each recipient: the number of days to the first appearance of a given antibody and to its maximum titre, the titre at the 14th or 15th day after first injection, and the highest titre regardless of time.

3. RESULTS

(i) *The antigenicity of the experimental antigens and the general antibody response curves*

The recipients were so selected that most of them lacked all the three experimental red cell antigens, M, V and U′, and all except two of the twin pairs lacked at least two of these antigens. The twins made a random sample of the cattle popu-

Table 2. *The antibody response to the antigenic factors V, M, and U′ and the concordance within pairs*

	Antibodies		
	V	M	U′
1. No. of MZ pairs where antibody was expected:	25	25	24
Both twins produced antibody	13	23	23
One twin produced antibody	3	0	1
No twin produced antibody	9	2	0
Concordant pairs (%)	88·0	100·0	95·8
2. No. of DZ pairs where antibody was expected:	7	8	5
Both twins produced antibody	1	7	4
One twin produced antibody	4	0	0
No twins produced antibody	2	1	1
Concordant pairs (%)	42·9	100·0	100·0
3. Pair 205–206			
Expected antibodies:	1	—	1
One twin produced antibody	0	—	1
No twin produced antibody	1	—	0
4. Percentage response	53·0	90·9	93·3

lation of Sweden. Even though relatively small, the material should therefore give a fairly good picture of the general antibody response against the V, M and U′ antigens employed. The latter antigenic factor yielded the best response. No less than 93·3 % of the recipients lacking the U′ antigen produced anti-U′. The corresponding frequencies for anti-M and anti-V were 90·9 and 53 % (Table 2). In Table 3 more detailed information is given on the antibody response in those animals which produced detectable amounts of a given antibody at least once during the experimental period. There were marked differences between the antibody populations.

Anti-U′ appeared first on the average 11·4 days after the first injection, and its maximum titre was the highest of the three. The M-antibodies appeared on the average 4 days later and the average maximum titre was 1·5 units lower. The V-antibodies were produced at a slower rate and the average maximum titre was only 3·5 units. The average response curves for each of the three antibody populations (Fig. 1) show the same general picture. However, in many cases those animals which reached their maximum titre early had lower maximum and less persistency. As a result the peaks of the average response curves are lower and appear later than the average maximum titres in Table 3.

The information which could be obtained on the anti-W was limited. However, anti-W seems to be easily produced. All ten recipients without the W antigen produced anti-W and the titre was of almost the same strength as the anti-U′ occurring in the same sera.

No less than twenty-six of the seventy-two recipients produced some antibodies of unknown specificity. These antisera reacted thus with cells lacking any of the known antigenic factors against which antibody production could be anticipated. The unspecific antibodies were generally weak and sporadic. In seven individuals only, such antibodies occurred in three or more successive samples. It was not possible to isolate any unspecific antibodies by absorption from the stronger antibodies of known type in the sera.

(ii) *The genetic influence on antibody response*

As stated in the section on Material and Methods, it was necessary to base the present study on twins which were or had been used in studies of the effect of certain differences in feeding and management on general productivity. In order to estimate the genetic influence on antibody response the possible effect of experimental treatment should be accounted for. The composition of the data allowed studies of the influence of age and feeding level only. However, the other treatments (e.g. interval between milkings) can hardly be expected to have affected the antibody response.

(iii) *The age influence*

All experimental animals were at least 12 months old and had thus passed puberty when the immunizations were started. The proportion of the animals lacking the M or U′ antigens which produced anti-M or anti-U′ was above 90 %. No difference existed between the different age classes with regard to the total percentage of response for the entire experimental period. However, there were rather marked differences between age classes with regard to the rate at which the antibodies appeared; 15 days after the first injection 45 % of the animals below 18 months of age had detectable amounts of anti-M in their sera, while the response among the mature animals (34–93 months) was 82 % (Table 4). The corresponding figures for anti-U′ were 69 and 90 %. The total response of anti-V was much less and no systematic age effect was indicated.

The intensity of the antibody response in animals which produced detectable amounts of antibody during the period of study appeared also to vary with age,

Table 3. *Characteristics of antibody production against the V, M and U′ antigen**

	Antibody type								
	M			U′			V		
Characteristics	No. of animals	Mean	Standard deviation	No. of animals	Mean	Standard deviation	No. of animals	Mean	Standard deviation
No. of days to 1st antibody	60	15·02	7·53	56	11·43	2·58	35	16·31	6·19
No. of days to highest titre	60	21·90	8·18	56	20·66	9·53	35	23·26	8·80
Titre at 15th day	60	3·17	2·95	56	5·09	2·63	35	1·80	1·91
Highest titre	60	5·38	2·04	56	6·91	2·02	35	3·51	1·62

* The data refer to those recipients only which produced detectable amounts of the respective antibody at least once during the experiment.

Table 4. *The influence of age on antibody response*

	Anti-M response (%)				Anti-U′ response (%)				Anti-V response (%)			
Age (months)	No. of animals	To 15th day	To 25th day	During whole period	No. of animals	To 15th day	To 25th day	During whole period	No. of animals	To 15th day	To 25th day	During whole period
12–18	20	45·0	75·0	90·0	16	68·8	87·5	87·5	20	30·0	40·0	50·0
19–24	24	62·5	87·5	91·7	24	95·8	100·0	100·0	24	41·7	62·5	66·7
34–93	22	81·8	86·4	90·9	20	90·0	90·0	90·0	22	27·3	40·9	40·9

particularly with regard to anti-M. All the characteristics used to measure the anti-M response differed significantly between age groups (Table 5). It took a longer time before anti-M was produced and the titre was on the average much lower in the younger group (12–18 months) than among the mature animals. The influence of age on anti-V was also marked, while no effect on anti-U′ was encountered. Even though the present data did not allow detailed studies of the age effect, this factor had apparently to be considered in the genetic analyses.

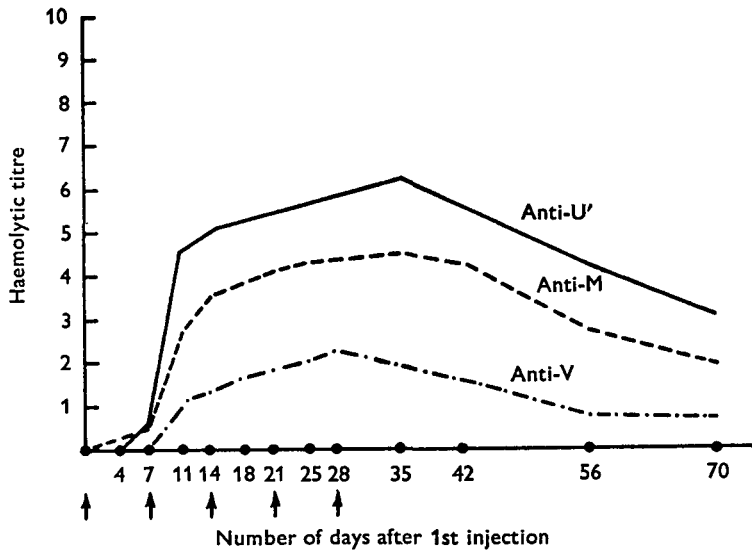

Fig. 1. Average anti-U′, anti-M and anti-V titres in recipients which produced the respective antibodies during the experimental period. All DZ twins and the twin with the lowest number of each MZ pair are included. The arrows indicate the days of injection and the points the days of serum collection.

(iv) *The effect of feeding level*

Some twin partners were split between different feeding levels. One of the twins in each of thirteen pairs (five MZ and eight DZ) got a standard ration while the respective co-twins got 60 or 80% of this amount up to 24 months of age (cf. Table 1). Ten such pairs lacked the M antigen and 8 and 11 had no U′ and V respectively. There was no difference in the percentage of response between feeding levels for any of the antibody types. However, when the intensity of antibody production was measured (same characteristics as in Table 5) there was a slight indication of higher antibody response in the normally fed than in the underfed animals. However, the difference reached statistical significance only for anti-V and the number of days to the maximum titre.

(v) *The similarity between co-twins*

The concordance within pairs with regard to the presence or absence of a specific antibody was complete for anti-M. One MZ pair and the twins 205–206 were dis-

Table 5. *The influence of age on the intensity of antibody production in animals which produced detectable amounts of antibody during the period of study*

Characteristics	Anti-M			Anti-U′			Anti-V		
	Age of animals (months)			Age of animals (months)			Age of animals (months)		
	12–18	19–24	> 33	12–18	19–24	> 33	12–18	19–24	> 33
No. of animals	19	22	20	14	24	18	10	16	9
No. of days to 1st antibody	18·9	16·0	10·5	12·3	11·6	10·6	18·7	15·7	14·8
No. of days to highest titre	26·9	21·1	18·5	20·6	21·0	22·6	25·4	21·7	23·7
Titre at 14th or 15th day	1·8	2·1	5·6	4·6	5·4	5·0	1·2	2·3	1·6
Maximum titre	4·7	5·0	6·5	6·9	7·0	6·8	2·8	3·9	3·6

Table 6. *The titres and reaction pattern of antisera from two MZ pairs (5–6 and 105–106) collected 14 days after the first injection*

Test cells \ Antiserum dilutions	Twin no. 5								Twin no. 6								Twin no. 105						Twin no. 106						Pertinent antigenic factors on the test cells
	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/2	1/4	1/8	1/16	1/32	1/64	1/2	1/4	1/8	1/16	1/32	1/64	
227	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F F . . .
276	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F F . . .
432	2	3	1	1	0+	.	.	.	2	2	1	1	0+	.	.	.	2	2	tr	.	.	.	1	1	tr	.	.	.	F F M . .
64	0+	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F V . . .
190	0+	.	.	.	.	.	.	.	0+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F V . . .
994	0+	.	.	.	.	.	.	.	0+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F V . . .
996	tr	.	.	.	.	.	.	.	0+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F V . . .
260	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	W F F . . .
265	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	W F F . . .
788	4	4	4	4	3	.	.	.	4	4	4	4	3	.	.	.	1	1	.	.	.	.	1	0+	.	.	.	.	F F . U′ .
808	4	4	4	4	3	tr	.	.	4	4	4	4	4	tr	.	.	2	tr	.	.	.	.	2	tr	.	.	.	.	F F . U′ .
45	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	W V V . . .
385	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	W V V . . .
344	4	4	4	2	tr	.	.	.	4	4	4	2	tr	.	.	.	4	4	2	2	0+	.	2	2	1	0+	.	.	W V V M . .
55	0+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	W F V . . .
191	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	W F V . . .
232	4	4	4	4	2	.	.	.	4	4	4	4	4	1	.	.	tr	.	.	.	.	.	2	0+	0+	.	.	.	W F F . U′ .
269	4	4	4	4	1	.	.	.	4	4	4	4	2	.	.	.	tr	.	.	.	.	.	1	.	.	.	.	.	W V V . U′ .
354	4	4	4	4	1	.	.	.	4	4	4	4	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	W V V . U′ .
156	4	4	4	4	4	2	tr	.	4	4	4	4	4	1	.	.	4	4	4	3	1	.	4	4	3	3	tr	.	W F V M . .

cordant for anti-U′ (cf. Table 2). The similarity was less for anti-V: in three MZ and four DZ pairs one twin only produced anti-V. The degree of concordance gives only a rough indication of the similarity between twins with regard to antibody production. The reactive patterns of twin sera tested against many different cells and the response curves for successive samples from the same individuals tested against single blood samples were therefore studied (Figs. 2–4). In Table 6 details are given on the reaction scores obtained with sera from two typical MZ pairs sampled 14 days after the first injection and tested against twenty different cell samples. Both pairs lacked the M, U′ and V antigens. The strength of anti-M was approximately the same in the two pairs, while the anti-U′ titre was much higher in pair 5–6 than in 105–106. At this stage the animals had produced very weak anti-V only. The co-twin sera gave rise to almost identical reactive patterns.

The U′ cells were heterogeneous with regard to reactivity. Some cells, e.g. 269 and 354, ceased to react one serum dilution earlier than the other U′ cells, 788, 808 and 232. This difference was repeatable in tests with sera from twenty-six different animals. The results therefore suggest a heterogeneity of the U′ antigen. In the titre curve in Figs. 1, 3 and 4 one blood of strong U′ activity was used as test cell. The use of a U′ cell from the weaker group would have decreased the level of the titre curves by approximately one unit, though the shape of the curves would have remained the same. Serologic subtypes indicating minor differences in the reactive substances have been found for many red cell antigens in cattle (e.g. Stormont, 1950; Grosclaude, 1965). Though of minor magnitude the differences in the U′ antigen found here probably also reflect small genetic differences in the antigen.

Figures 2–4 show the antibody titres in successive samplings from twins in three different categories (MZ, DZ and two exceptional pairs). Most MZ twins had remarkably similar titre curves, while the difference was large between pairs. In pair 5–6 the anti-U′ titre was high as early as 11 days after the first injection and the maximum titre was reached at 14 days (Fig. 2). Pair 211–212 had not yet produced any detectable anti-U′ at the time when 5 and 6 reached their maximum titres. However, pair 211–212 ultimately reached a higher titre than 5 and 6. After its peak the antibody titre descended much faster in 211–212 than in 5–6. In the latter pair it took approximately 36 days before the maximum titre was halved as compared with 25 days in 211–212. The same general pattern was obtained also for anti-M. The twins 5 and 6 produced anti-M shortly after the first injection. The maximum was reached as early as at 11 days and the antibody production was relatively persistent. In pair 211–212 the M-antibodies were produced late, though the titre became high. Persistency was low, however.

In most of the MZ pairs the twins showed very similar titre curves. However, there were a few minor exceptions (e.g. pair 105–06, Fig. 2) and one very marked exception. The titre curves for this latter pair (3–4) are given in Fig. 3 along with the curves for the pair of unknown zygosity (205–206). The small DZ group was more variable (Fig. 4). Some DZ twins gave rather or even very similar curves, while in other pairs the differences were large.

The titre curves give the general impression that the antibody response is very similar within MZ pairs and differs markedly between pairs. This applies not only to the amount of antibody but also to the rate at which the antibodies are produced. In order to get a quantitative measure of the degree of likeness the variances

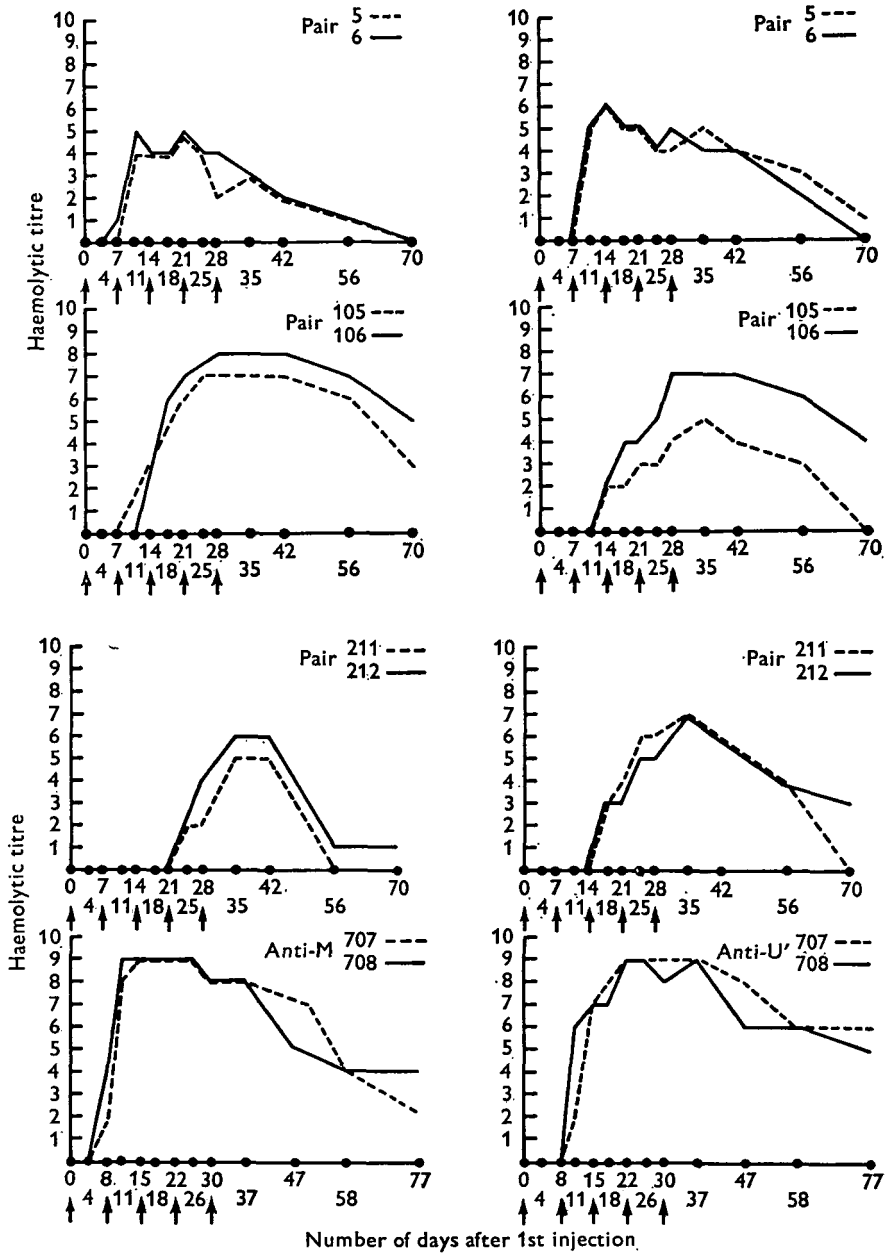

Fig. 2. The titre curves for anti-M (left) and anti-U′ (right) in some representative MZ pairs. The arrows and dots in this and subsequent figures have the same meaning as in Fig. 1.

https://doi.org/10.1017/S0016672300011460 Published online by Cambridge University Press

within and between pairs were estimated for each of the characteristics given in Material and Methods. In order to eliminate most of the effect of age and of sex (if any) on antibody response, the analyses were made within classes of bulls, heifers and cows (cf. Table 1). The results are summarized in Table 7. There were highly significant differences between MZ pairs for each of the four characteristics of the anti-U′ response and for 3 of the characteristics of anti-M and anti-V. The number of pairs was relatively small and somewhat variable results should therefore be expected. However, on the average, 55 % of the variation in antibody response appears to be ascribable to differences between MZ pairs. For the strongest antigen (U′) the between-pair component accounted for 68 % of the total variance within classes of pairs, and for the weakest antigen the corresponding figure was 48 % only.

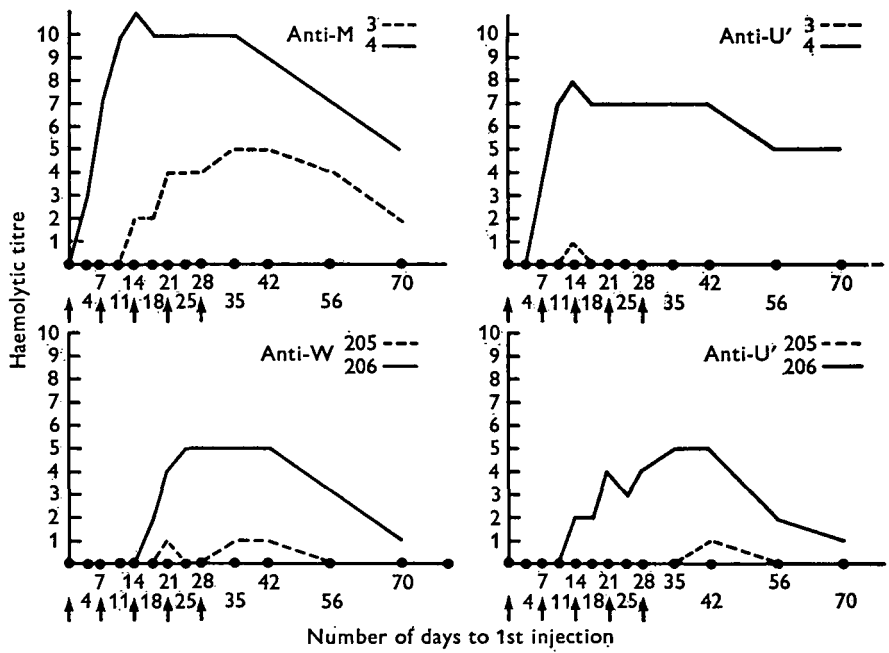

Fig. 3. The titre curves for anti-M and anti-U′ in the two exceptional pairs, 3–4 (MZ) and 205–206 (unknown zygosity).

There were seven DZ pairs in which both twins produced anti-M, and for these the differences between pairs were significant for one of the characteristics only. On the average the differences between pairs accounted for 33 % of the variance in four characteristics.

(vi) *The simultaneous response of different antibodies in the same animals*

Most of the recipients lacked at least two of the blood group antigens of the donor cells. It was therefore possible to study the simultaneous responses of two different antibodies in the same animals. The twin having the lowest number in each informative MZ pair, animal 205 and all informative DZ twins were included in the

study. For anti-M and anti-U′ the animals were grouped into two classes according to whether they had a maximum titre above or below the average maximum titre of all animals producing a given antibody in the entire period. The average maximum anti-M titre was 5·4. (Table 3) and the animals were accordingly divided into one group with anti-M titres of 5 or below and the other with titres above 5. The same system of classification was used for anti-U′. For anti-V the division was made into responding and non-responding animals only.

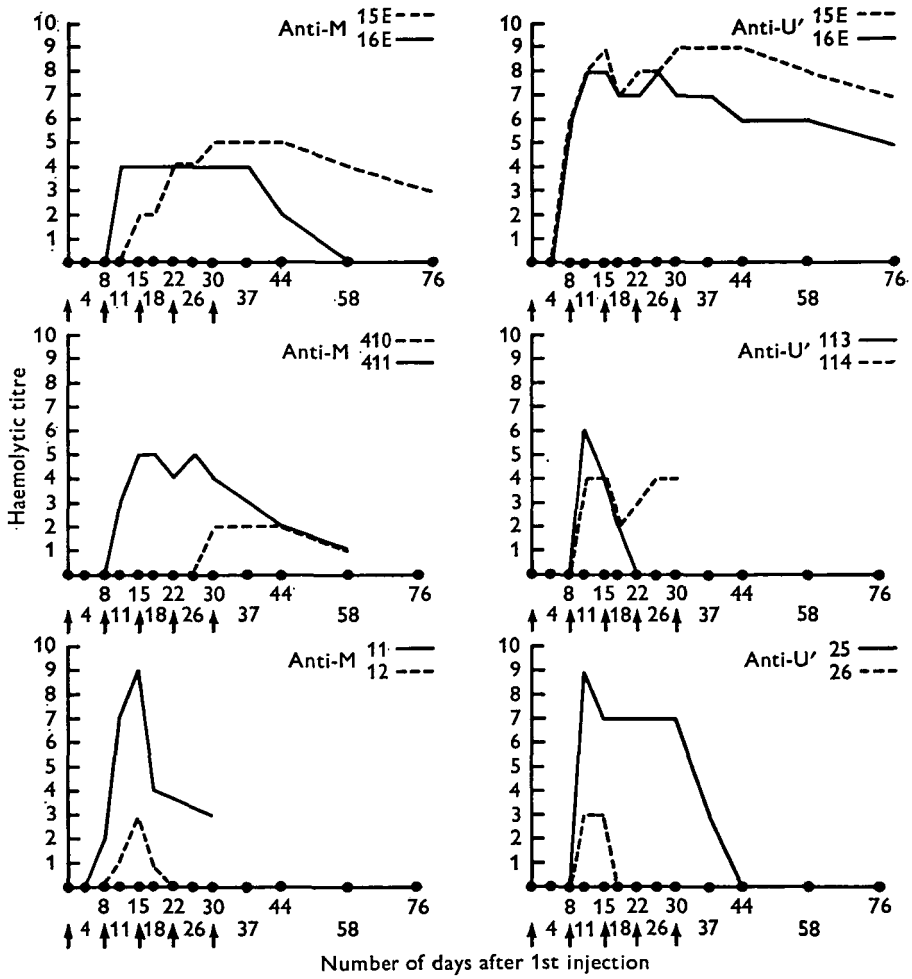

Fig. 4. Titre curves in four representative DZ pairs.

The results are summarized in Table 8. There was a significant positive association between the antibody responses of the three antibody types. Animals having a high maximum anti-U′ titre had also more often a high maximum anti-M. Similarly, those with a high maximum anti-M or anti-U′ produced more often anti-V than those having lower anti-M and anti-U′ titres. The overall results therefore suggest that there is a rather marked positive association between the in-

tensities of production of these three antibodies in a given animal. The variation in age between the various animals can hardly be the main cause of this association as the anti-U′ response and titre differed very little between age classes (Tables 4 and 5). The most plausible explanation of the association seems, therefore, to be a common control of the response of the three different antibodies.

Table 7. *The genetic influence on antibody production: the proportion of the variance within classes of MZ pairs (bulls, heifers and cows) which was due to differences between pairs*

Characteristics	Anti-M 23 pairs Total variance within classes	% between pairs	Anti-U′ 23 pairs Total variance within clases	% between pairs	Anti-V 12 pairs Total variance within classes	% between pairs
No. of days to first antibody	27·5	67**	4·9	54**	39·3	66**
No. of days to highest titre	65·7	58**	82·9	94**	86·7	2
Titre at 15th day	4·4	23	6·8	65**	3·0	64**
Maximum titre	3·6	51**	4·0	58**	0·8	60*

Significance of differences between pairs * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

Table 8. *The relationship between the maximum titres of anti-M and anti-U′ and the occurrence of anti-V in animals which were expected to produce at least two of these antibody types*

	Max. anti-M titre			Max. anti-U′ titre		
	⩽ 5	> 5		⩽ 7	> 7	
No. of animals with max. anti-M titres above 5	.	.		4	7	11
No. of animals with max. anti-M titres below 5	.	.		16	5	21
				20	12	32
				$\chi^2_1 = 4{\cdot}9$, $P < 0{\cdot}05$		
No. of animals with anti-V	8	9	17	6	9	15
No. of animals without anti-V	15	3	18	13	3	16
	23	12	35	19	12	31
	$\chi^2_1 = 5{\cdot}1$, $P < 0{\cdot}05$			$\chi^2_1 = 5{\cdot}6$, $P < 0{\cdot}05$		

4. DISCUSSION

Antibody response depends on an interaction between the injected antigen and pertinent cells in the recipient. Interaction phenomena are always difficult to analyse genetically. In the present study a standard dose of red cells from the same

https://doi.org/10.1017/S0016672300011460 Published online by Cambridge University Press

donor was injected into a number of twin pairs. The red cells carried three gene-controlled antigens (M, U′ and V) which were lacking in most recipients. The response against the U′ antigen was much stronger than against the other two antigens. It is commonly observed that the dosage of the injected antigen plays an important role in antibody formation. The amount of the three antigens on the cattle red cells is unknown. The strong anti-U′ response might naturally have been caused by a higher amount of U′ on the red cells. However, previous experience with much larger dosages of blood carrying either M, U′ or V (e.g. 50 ml of blood: Rendel, 1958) indicates that the larger dosages do not give a higher response and that the relative difference between the three antigens remains approximately the same. The difference in average antibody response between the three antigens employed is therefore probably due to a difference in general antigenicity so that the U′ antigen is more antigenic than M and V.

The twin pairs showed a very wide variation of antibody response to each of the antigens (Figs. 2–4). Some animals gave a very fast response, while others produced their maximum titres when the former had almost ceased to show antibodies. It therefore seems likely that the recipients got sufficient antigenic stimuli and that the response was mainly governed by other factors than the dosage.

More than 90 % of the recipients lacking the M and U′ antigens produced the corresponding antibodies. At first sight the ability to produce anti-M and anti-U′ might therefore appear almost as if determined by a single recessive gene, i.e. response occurred in animals lacking the dominant genes for the M and U′ antigens respectively. Cinander (1960) suggested also that the intensity of antibody response may be a function of the presence or absence of autologous antigens related to the injected antigen. The difference between the donor and recipients of the present study was limited to single gene-controlled entities and such a mechanism can therefore hardly explain the quantitative variation in the anti-M, anti-U′ or anti-V.

The differences between MZ pairs accounted for approximately 55–60 % of the quantitative variation in antibody response. It is always difficult to estimate heritability from MZ twin data (for discussion see Donald, 1958; Rendel, 1963). In the present study the between-pair component of variance seems rather to underestimate than overestimate the genetic influence. The environmental differences within pairs were larger than between animals kept under ordinary managerial regimes. Furthermore, the analyses had to be based on those animals only which responded against a given antigen. The pairs which did not respond were alike but could not be included as they showed no antibody. Studies by Neimann-Sørensen (1958), Rendel (1958) and others indicate that certain animals require re-immunization before they respond to the red cell antigens employed here. The non-responding pairs might thus have responded if subjected to re-immunization.

Studies of monozygous twins always involve a component of uncertainty as there is no exact method of diagnosis. Monozygosity cannot be proved, only disproved. In eighteen of the twenty-six MZ pairs the co-twins were extremely alike with regard to the shape of the antibody response curves. In six pairs the antibody

curves showed a high degree of likeness, even though for a shorter or longer period the curves of some partners deviated somewhat (e.g. pair 105–016, anti-U′, Fig. 2). Finally, there were two exceptional pairs. The twins 31–32 were both expected to produce M, U′ and V antibodies. Actually, neither developed anti-V or anti-M (very alike in this respect). No. 32 produced anti-U′ while No. 31 did not. This was thus the single discordant MZ pair with regard to the presence or absence of anti-U′ (cf. Table 2). However, the response curve of No. 32 deviated markedly from the average anti-U′ curve (Fig. 1). Anti-U′ was not produced until the 35th day and the maximum titre value was 3 only. The results of pair 3–4 were yet more controversial. The animals were 3 years old when subjected to immunization. No. 4 produced anti-V and weak antibodies of unspecific type, while no. 3 produced neither. Both twins developed anti-U′ and anti-M, but no. 4 had much higher titres than her co-twin (Fig. 3). Actually no. 4 showed the highest anti-M titre in the whole study. Repeated diagnoses by use of blood groups, biochemical markers (transferrin, postalbumin and alkaline phosphatase) and morphology did not give any evidence of dizygosity. The twins were close to 5 years when they left the herd and no alarming differences had been noticed in the yield and composition of the milk, in body size or in any other character. The simplest explanation of the large differences of antibody response in the twins 3 and 4 would naturally be dizygosity. The exclusion of the pair 3–4 from the MZ group would increase the variance component between MZ pairs to 70 and 80 % for the anti-M and anti-U′ response respectively. However, we do not feel that there is any independent evidence which would allow the exclusion of this pair from the MZ group. Alternative explanations should therefore be tried.

The occurrence in one of the twins of a somatic mutation in the stem cells of lymphocytes or plasma cells might explain the difference in antibody response of the twins 3 and 4. Studies by Stone, Friedman & Fregin (1964) on chimaeric dizygous cattle twins have shown that the proportion of the red cells in the admixture may change markedly in a relatively short period of time and in one case an entirely new cell type (possibly arisen by somatic cell mating) became predominant. By analogy a somatic mutation in the stem cells of the antibody-producing cell systems may cause large differences between MZ twins in their capacity to produce antibody.

Most of the recipients of the present study lacked two or more of the antigens present on the donor's red cells. So it was possible to study the simultaneous response of two or more antibodies in the same animal. There was a positive association between the titres of the two or more antibodies elicited in the same animal. This result agrees with those obtained by Carlinfanti (1948) in man, by Sobey & Adams (1961) and others in rodents and also with the more recent results on polypeptide conjugates (Benacerraf, 1965). In addition there was also a similarity between the titre curves obtained for the different antibodies in the same animal or MZ pair (Fig. 2). The intensity of the antibody response against specific gene-controlled entities such as the red cell antigens seems to be under close genetic control. The similarity in the simultaneous response to different antibodies

suggests, therefore, the existence of genetic variation in the general antibody-producing ability or at least in the efficiency of the antibody response against a whole array of more or less related antigens.

SUMMARY

1. The antibody response was studied in twenty-five monozygous (MZ) and ten dizygous (DZ) pairs of cattle twins, which were inoculated with red cells from one cattle donor carrying the M, V and U′ antigens. These red cell antigens are controlled by genes at three different loci. Most of the recipients lacked two or all of these antigens. All had passed puberty at the onset of the experiment.

2. The antibody response against the three antigens differed markedly. Anti-U′ appeared on the average 11 days after the 1st injection and the maximum titre values were high. The V antibodies were produced at a slow rate, while anti-M was intermediate.

3. There was some influence of age on the rate and intensity of antibody production. Older animals gave a faster response and, with regard to anti-M, also higher titres.

4. Twins belonging to the same MZ pair usually produced very similar antibody curves. However, there was at least one noticeable exception, which is given special consideration in the Discussion. The differences between MZ pairs accounted for a considerable portion of the variance in the different measures of antibody response and in most cases this portion remained significant also after the elimination of the influence of age and sex. The DZ pairs were more variable.

5. The simultaneous response of two or three antibodies in the same animal was studied. There was a significant positive association between antibody types which suggests the existence of individual (probably genetic) differences in the general antibody-producing ability.

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