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BLOOD GROUPS IN BOVINES. II. NORMALLY OCCURRING ISOANTIBODIES IN CATTLE BLOOD

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The antibodies which are used to detect red-cell antigens (blood factors) may be either " normally occurring " or " immune " in origin. The normally or naturally occurring antibodies formed the basis on which the universally recognized A B O blood groups of man were first worked out by Landsteiner (1901).

After the first blood groups in man had become known a search was made for the presence of red-cell antigens in cattle and other animals. The results were rather limited at first because most of the blood group workers searched mainly for normally occurring antibodies and for similarities between human and animal blood factors (Mourant, 1954). Greater progress was made when these workers started to produce immune antibodies for better and more complete differentiation of red-cell antigens. To date more than 40 different types of immune antibodies could be produced for the detection of their corresponding antigens, which are arranged in at least ten blood group systems according to their genetic relationship (Osterhoff, 1960).

Among these blood group systems the J system merits special attention. Stormont (1949) demonstrated that the J substance of J positive cells is primarily a constituent of the plasma and only secondarily attached to the erythrocytes. Furthermore, it is the only red-cell antigen of bovine blood which is detected by normally occurring antibodies. It was believed until recently that anti-J occurred only naturally, but Tolle (1960) described the production of anti-J by hetero-immunization in rabbits. The anti-J is not only the most frequently occurring isoantibody in cattle serum but is also related to normally occurring antibodies in other species. Neimann Sørensen, Rendel & Stone (1954) were able to show that cross-reactions existed between cattle J, sheep R and human A antigens. Stone & co-workers (1954, 1956, 1957, 1958) performed the most extensive studies on the J system and were able to show the inheritance of the three distinct phenotypes of the J system: J^{cs}—cattle with J substance on the cells and in the serum; J^{s}—those with J substance only in the serum, and J^{a}—those without J substance but whose sera may contain anti-J. This paper will deal with the third group and represents mainly the study of the seasonal variation in the occurrence of natural or normal isoantibodies.

Stormont (1949) was the first to describe a marked variation in the titre of the isoantibodies against J among individuals and a periodic variation in any particular animal. Forschner (1955) studied 4,370 different sera and stated that the frequency of occurrence of different hemagglutinins among bovines was about 10 per cent higher in summer than in winter. Furthermore, he was able to confirm the variability of the isohemagglutinin titres found by Tolle (1953) and other German workers and thought that the titre variations were connected with pregnancy and feeding.

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Rendel, Tolle & Neimann Sørensen (1957) were able to prove that the seven types of isohemagglutinin isolated by the German workers correspond with immune hemolysins described by the American workers, and that the most important anti-A of the former group was identical with the naturally occurring anti-J of the latter group. Stone (1956) studied the seasonal variation most carefully and found the highest titres during the late summer and early autumn, and the lowest titres during the late autumn and early winter. Braend (1959) confirmed these findings for animals in the northern hemisphere.

Since no data were available for bovines in the southern hemisphere it was decided to study possible seasonal titre changes in South African breeds. Furthermore, an experiment was performed in an attempt to collect more information on the physiological basis for the seasonal variation in the level of normal antibodies.

A.—The Seasonal Variation in the Level of Normally Occurring Isoantibodies

The first experiment was started in January 1958 with 90 animals selected at random: This number was composed of 30 Friesian cows, 30 Afrikaner cows and 30 Afrikaner × Hereford oxen.

Blood samples from the jugular vein were taken between the fifteenth and twentieth day of every month into clean, dry 15 ml. bottles, and were allowed to clot. The clotted samples were incubated in a water bath at 37° C for 20 minutes, and then were allowed to stand for about two hours at room temperature. The sera were poured off, centrifuged until clear and stored in a deep-freeze cabinet, at −79° C. The hemolytic tests were performed according to the method described by Osterhoff (1960). In the present studies, successive twofold dilutions of each sample of serum (1/1, 1/2, 1/4 – – – 1/512) were made with saline using a Cornwall Pipetting Outfit (Becton, Dickinson & Company). Each serum, in ten dilutions, was tested against bovine erythrocytes of six different animals. The six different red cells were selected according to the reactivity against anti-J, two strongly and two medium reactive cells and two non-reactive cells, and were used throughout for all tests recorded in this paper. The test readings were made after half-an-hour, two hours and after another interval of two hours, and were recorded in the usual manner as 4 (complete hemolysis), 3, 2, 1, trace and 0 (no hemolysis). The scores were calculated in a manner similar to that described by Stone (1956). The corresponding scores to the readings were 5, 4, 3, 2, 1 and 0, and the sum of the scores (total score) given to the reaction in each tube of the titration was recorded as the strength or level of the antibodies. For example, a serum of which the reactions are recorded at the final readings as 4 up to a dilution of 1: 64, 2 at a dilution of 1: 128 and trace at a dilution of 1: 256, would have a total score of $35 + 3 + 1 = 39$.

The following results were obtained: of the 90 animals in the experiment 42 had normally occurring antibodies in their serum, 14 of them being Friesians, ten Afrikaner cows and 18 Afrikaner crossbred oxen. A careful study of the results revealed that not all normal antibodies were anti-J. The serum of one of the Afrikaner cows contained a high level anti-V and that of four oxen a fairly high level of anti-Y_2. Normally occurring isoantibodies against the Y_2 antigen have never been described before. As a result of a series of comparative tests between sera containing normally occurring anti-Y_2 and those with anti-Y_2 produced by immunization, it was decided to use normal Y_2 antiserum as reference reagent in routine blood group determination tests. One of the Friesians displayed an antibody-type in its serum which could not be identified. Unfortunately this animal was used by accident as recipient for the production of immune antibodies of the A^1, C, X_2

and L type, and had to be excluded from the breed comparison of the concentration of normally occurring isoantibodies. The variation in the occurrence of the above listed antibodies for the first year of the experiment is given in Fig. 2. The results are based on the scores with only one of the strongly reactive cells used in the titrations. This cell carried, among others, the antigenic factors Y_2, V and J and reacted therefore with all normally occurring isoantibodies discussed here. For each group of animals the averages of total scores are given for each month irrespective of the antibody type involved.

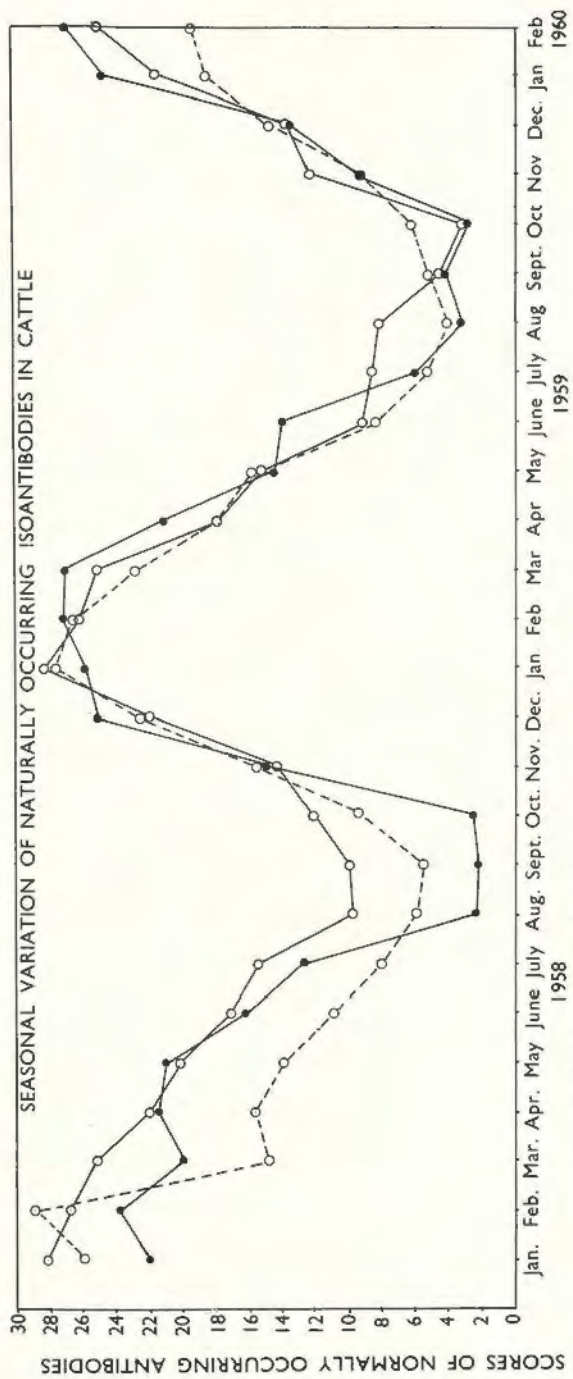
At the end of 1958 some of the animals (three Friesians and one Afrikaner) were taken out of the experiment and had to be excluded from further studies. Furthermore 12 oxen were removed from the whole group (four with normal anti-Y_2, the rest with normal anti-J) for a special experiment which will be mentioned later. The remaining animals were used for the calculation of average values of the total scores for the normally occurring J-antibodies. The Afrikaner cow with anti-V is also excluded from the average values given in Fig. 1. Using the two strongly reactive cells and taking the average of two values for every animal every month the curves shown in Fig. 1 were obtained.

The variation of the normal V and Y_2 antibodies is given in Table 1. Only one strongly reactive cell with anti-V and anti-Y_2 was used for the calculation of the total score. In the same table the total score for the animal mentioned before (cow No. 8498 produced anti-C by immunization) is also given.

TABLE 1.—*Seasonal variation for normally occurring isoantibodies other than anti-J*

Year/Month	Normal anti-V (one animal)	Normal anti-Y_2 (four animals)	Normal anti-Y^1 (?) (one animal)
1958 January	18	38·5	23
February	25	46·5	14
March	—	43·8	15
April	—	40·0	0
May	26	40·8	0
June	11	36·0	40 (immune C-antibodies)
July	31	31·8	33
August	23	27·8	26
September	24	25·8	26
October	29	31·3	34
November	25	39·9	40
December	26	39·0	42
1959 January	30	38·0	39
February	30	38·5	31
March	35	39·8	36
April	22	35·0	34
May	30	37·8	39
June	28	31·3	33
July	25	26·3*	21
August	21	24·8	19
September	25	26·3	20
October	21	26·0	50
November	30	27·5	41
December	35	29·0	36
1960 January	30	28·0	32
February	30	32·3	35

* From July all four animals were included in a Dark–Light experiment which will be described later.


SEASONAL VARIATION OF NATURALLY OCCURRING ISOANTIBODIES IN CATTLE
SCORES OF NORMALLY OCCURRING ANTIBODIES
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
Jan. Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan Feb Mar Apr May June July Aug Sept. Oct Nov Dec. Jan Feb
1958
1959
1960
= Afrikaner (average of 8 animals)
= Oxen (average of 6 animals)
= Friesian (average of 10 animals)

The seasonal variation of these antibodies is not as clear as in the case of anti-J because the average of the three groups of the latter is based on greater numbers. The variation of the immune C-antibodies is very interesting and also the fact that these antibodies remained for more than two-and-a-half years up till the present in the serum of that particular animal.

Immune antibodies usually disappear after two to six months and therefore, it is believed that the immune anti-C had a stimulating effect on the normal antibodies with the result that both types together gave the high level and the variation of antibodies as shown in Table 1. In the first three tests of the series fairly strong normal antibodies could be demonstrated which paralleled immune anti-Y^1 very nearly but not exactly. Already in April, in the fourth test, these antibodies had disappeared and the animal was used for immunization, as previously mentioned. The normal antibodies which were previously present in the serum were then dominated by immunization in such a way that they could not be recognized anymore.

In regard to Fig. 1 it should be mentioned that all animals in the two female groups were older than four years while the oxen in the third group were two-and-a-half to three years old at the start of the experiment. No variation studies have been performed on animals younger than two-and-a-half years of age. The eight Afrikaner cows mentioned in Fig. 1 gave birth to nine calves during the whole period. The ten Friesian cows calved 12 times in all, but the effect of reproduction cannot be seen in Fig. 1 at all because the calvings were spread over the whole period of 26 months and the smoothing effect of averaging obscured the small drops in the total scores of antibodies which occasionally occurred shortly after calving. These drops were not observed in all cases as samples were taken about a month after calving in some cases, and in other cases it could be shown that no decline in the titre of antibodies occurred.

An examination of the variation in Fig. 1 and Table 1 allows the following conclusions to be drawn:—

(*a*) There is no difference among breeds in the seasonal variation of naturally occurring antibodies.

(*b*) There is no difference between sexes in this seasonal variation.

(*c*) The feeding had no influence on the seasonal variation, because the Afrikaner cows and the Afrikaner cross oxen were in the veld without any extra feeding whatsoever, while the Friesians were fed throughout the year on green lucerne. (In South Africa some farmers with land under irrigation are fortunate in having green lucerne all the year round.)

From a summary of the results, it may be concluded that the concentration of anti-J varies in the same way as it has been found to do in the northern hemisphere. In all groups the highest titres were found during the late summer (January to February) and the lowest titres were found during the late winter (August to September).

B.—The Variation of Normally Occurring Isoantibodies in Relation to Climatological Data

In an attempt to obtain more information on the physiological basis for the striking seasonal variation in the concentration of normal antibodies the following climatological data have been collected:—

(1) Monthly maximum temperature at Onderstepoort (O.P.).

(2) Monthly sunshine hours observed at O.P. and calculated by the Weather Bureau, Pretoria.

(3) Monthly illumination values received from the Council of Scientific and Industrial Research (C.S.I.R.), Building Research Institute.

(4) Monthly radiation figures supplied by the Weather Bureau, Pretoria.

These values are tabulated together with the seasonal variation of the normally occurring isoantibodies in Table 2, and Fig. 2 represents graphically the values for maximum temperature and radiation in relation to the average titre scores for the three groups.

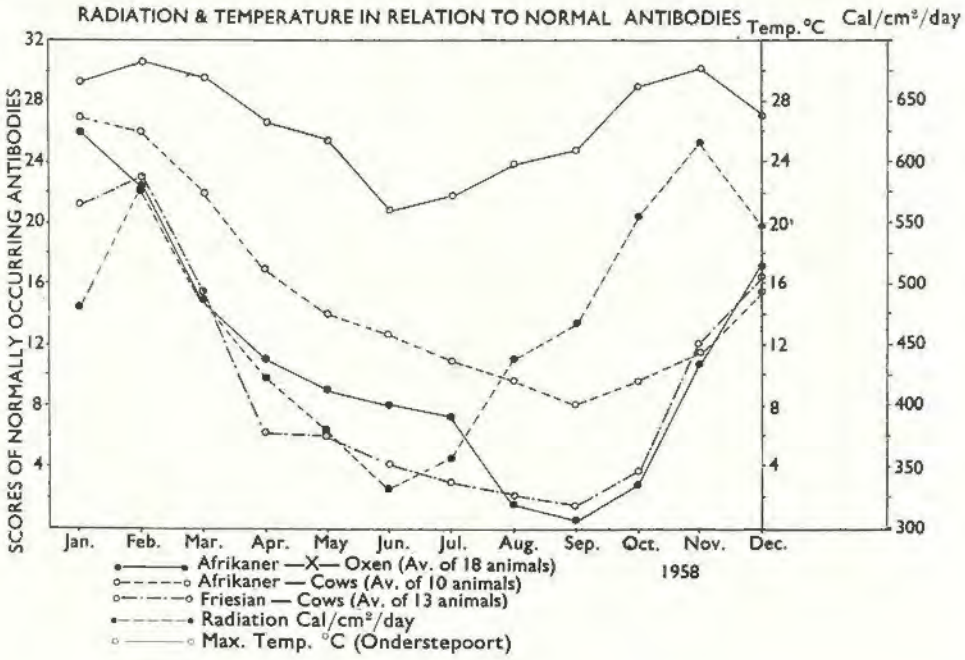


TABLE 2.—*Comparison of some climatological factors and naturally occurring iso-antibodies*

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Average Max. Temp. (°C)—												
1958 O.P. Laboratories.	29·3	30·5	29·6	26·6	25·4	20·9	21·9	23·9	24·9	28·9	30·1	27·0
Average Sunshine Hours (hours per day)—												
1958 O.P. Laboratories.	6·68	8·90	7·80	8·13	7·86	7·59	8·26	8·66	7·34	9·33	9·39	8·47
Average of five years, 1954–58 incl.	7·79	7·98	7·89	8·30	7·89	7·97	8·26	8·95	8·32	8·63	8·75	8·29
Average Sun and Sky Illumination Total (lumen/sq. ft.)												
1952–1954 C.S.I.R.												
Hourly values at—												
10 a.m.	11,523	11,246	8,748	6,752	5,381	4,383	4,196	5,397	7,312	9,474	7,063	11,465
1 p.m.	14,827	14,281	11,422	9,427	7,934	7,084	6,676	7,991	9,870	12,243	13,686	14,325
4 p.m.	9,054	8,194	6,078	4,402	3,155	2,830	2,785	3,576	5,075	6,800	8,065	8,831
Average Radiation (cal./cm.^{2} per day)	481·5	577·5	493·8	423·6	377·5	337·2	356·2	437·1	470·5	556·9	615·7	549·7
1958 Roodeplaat.												
Average titres for normal antibodies (score values):												
1958 Afrikaner (10 animals)	27·0	26·0	22·0	17·0	14·0	12·6	10·8	9·8	8·0	9·5	11·5	15·6
1958 Friesian (13 animals)	21·2	23·0	15·0	6·4	6·0	4·2	3·0	2·0	1·4	3·6	12·0	16·6
1958 Afrikaner oxen (18 animals)	26·0	22·5	15·0	11·0	9·0	8·0	7·2	1·4	0·6	2·8	10·8	17·4

The following comments on these data can be made:—

(*a*) The average maximum temperatures at Onderstepoort show about the same trend as that of the titre scores for the normal antibodies, but the lowest temperatures occurred in June while the lowest titre scores for all three groups were found in September.

(*b*) The variation in the sunshine hours is very small and it appears to be unrelated to the variation in the titre scores.

(*c*) The illumination presents difficulties because the measurements have to be taken under cloudy and clear conditions of the sky. (In Pretoria: 10 per cent cloud over, 40 per cent cloudy, 50 per cent clear). Clear differences exist between summer and winter but it is believed that these differences are not the important reason for the variation discussed here.

(*d*) The radiation figures (collected by the Weather Bureau at the Roodeplaat Research Station, only ten miles from Onderstepoort) show about the same variation as the titre scores for the antibodies. The lowest radiation values were observed during June and are comparable with the variation in temperature.

It seems, therefore, that the antibody production in the healthy animal is synchronized with the fluctuations in temperature and the radiation, but it is obvious that a retardation of about two to three months takes place in the formation of normal antibodies.

To prove this theory, some of the experimental animals were brought into a dark stable where it was relatively easy to exclude the radiation influence on the animals, more so than if one should try to exclude the temperature influence during several months.

C.—The Influence of Light on the Variation of Normally Occurring Isoantibodies

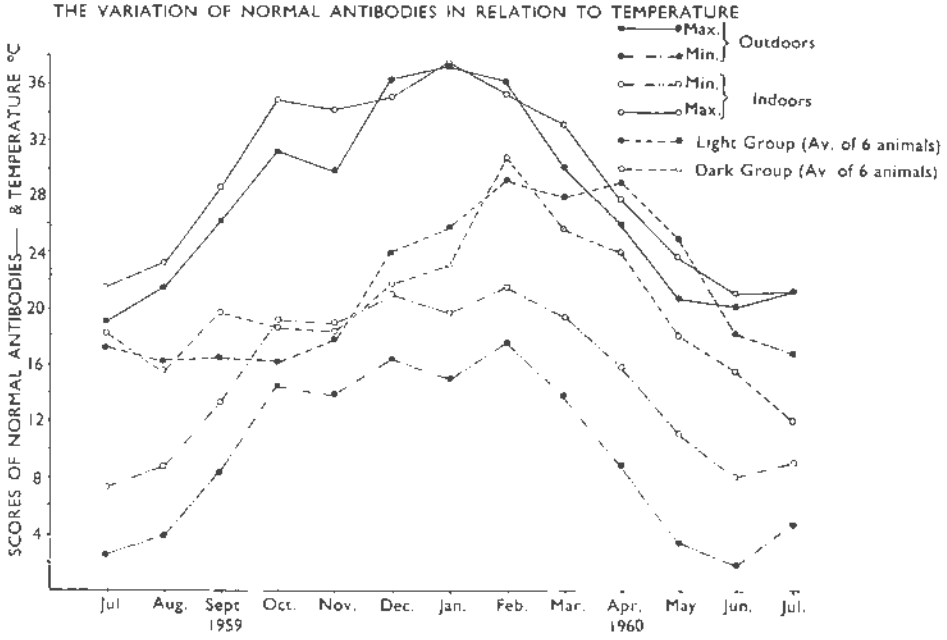
A light–dark experiment was performed on 12 oxen which were selected from the whole group of oxen in the previously described experiment. The 12 animals, then four to four-and-a-half years old, were placed in two groups according to the concentration of natural antibodies, two animals with anti-Y_2, and four animals with anti-J in each group. One group of six oxen was brought into an absolutely dark stable for exactly one year from 15 July, 1959 till 15 July, 1960. The other group remained outside as the control group. The oxen of the dark group were let out for exercise during the night between 9 p.m. and 3 a.m. During that time the stable was cleaned and forage brought in. Both groups received exactly the same ration throughout the whole time. Each animal was fed 5 to 6 lb. green lucerne, 10 to 11 lb. teff hay, $\pm$ 7 lb. maize silage and $\pm$ 6 lb. concentrate mixture with the necessary ingredients (vitamins, minerals, etc.). Two fans were installed to supply fresh air and to keep the stable as cool as possible. In order to have an exact comparison of the temperature and humidity both indoors and outdoors daily minimum and maximum temperature recordings were kept as well as readings of temperature and humidity both indoors and outdoors at 8.10 each morning (except Sundays). It must be emphasized that the six animals in the stable did not get any form of light throughout the whole time, even the blood samples were taken at night in the dark.

Besides the studies of normally occurring isoantibodies which were the main object of this experiment other samples were taken to obtain information in respect of the following:—

(1) Protein analysis of the serum.
(2) Red cell count.
(3) Red cell volume.
(4) Red cell size.
(5) Hemoglobin content.
(6) Erythrocyte sedimentation rate.
(7) Determination of coagulation time.
(8) Osmotic fragility test of erythrocytes.
(9) Blood sugar.
(10) Total protein.
(11) Skin biopsy for any changes of the skin.
(12) Hair thickness.
(13) Hair length.
(14) Hair composition.

Remarkably interesting results were obtained and will be discussed elsewhere because these observations have no direct bearing on the research work on blood groups or antibodies.

The hemolysis tests were performed in the same way as described before. To exclude possible errors in the evaluation of the degree of hemolysis and the corresponding scores under different test temperatures (Osterhoff, 1960) all tests were repeated at the end of the experiment. (One serum sample from each animal was stored at monthly intervals during the course of the experiment.)



The answer to the question, " does radiation or illumination play an important role in the antibody production? " may reasonably, therefore, be sought in the results presented in Fig. 3. The monthly minimum and maximum temperatures to which the two groups were subjected are given in Fig. 3 together with the group average of total scores for both types of isoantibodies.

The fact that the curves for the two groups are similar indicates that light (radiation, illumination) does not play a role in antibody production. Both the dark and the light group gave about the same results in respect to the variation of their normally occurring isoantibodies.

TABLE 3.—*The average variation of normally occurring anti-Y_2 and anti-J of the 12 animals before and during their subjection to the light and dark treatments*

Year/Month	Average scores of the naturally occurring antibodies			
	Light group		Dark group	
	Anti-Y_2 (two animals)	Anti-J (four animals)	Anti-Y_2 (two animals)	Anti-J (four animals)
1958 January	36·5	33·5	40·5	28·8
February	47·5	32·5	45·5	35·3
March	43·5	30·8	44·0	31·0
April	41·0	33·0	39·0	30·8
May	41·0	34·5	40·5	32·3
June	37·0	30·8	35·0	25·3
July	29·0	22·0	34·5	21·8
August	25·0	11·5	30·5	9·5
September	25·5	8·5	26·0	8·8
October	29·5	12·8	33·0	9·8
November	40·5	29·5	39·0	31·8
December	39·5	36·8	38·5	38·0
1959 January	38·0	32·5	38·0	37·3
February	39·0	31·5	38·0	34·8
March	39·0	37·0	40·5	36·3
April	35·5	29·5	34·5	34·8
May	36·5	22·5	39·0	23·8
June	30·5	15·3	32·0	20·3
July*	25·5	13·0	27·0	13·8
August	26·0	11·5	23·5	12·5
September	24·5	12·5	28·0	15·5
October	23·0	12·8	29·0	13·5
November	25·5	14·0	29·5	13·0
December	26·5	22·8	31·5	16·8
1960 January	28·0	24·8	28·0	20·5
February	31·0	28·5	33·5	29·5
March	25·5	29·3	31·0	23·3
April	29·0	29·0†	30·0	21·0†
May	22·5	26·3‡	26·0	14·0‡
June	21·5	17·0	25·0	10·8
July	18·0	16·3	20·0	8·0

* Commencement of the different treatments. From Jan., 1958 till July, 1959 the animals ran together under veld conditions.
† Very near the 5 per cent level of significance.
‡ Significant at the 5 per cent level acc. to Mann-Whitney test.

The antibody production in the reticulo-endothelial system, in the lymphocytes, plasma cells or wherever it does take place, is a complicated process, and it is believed that the stimulus comes from outside the animal body. The experiment was started in July when the concentration of antibodies is weak on the reasoning that if the radiation or other light effects do play any role, the concentration of antibodies in the dark group would remain at a lower level and only the concentration in the light group should increase in the usual way.

Table 3 gives further details of the total scores of the 12 animals throughout two-and-a-half years, one-and-a-half of these being prior to the commencement of the light (control) and dark treatments.

From Table 3 it can be seen that the variation of the concentration of all antibodies took place in about the same way as shown in Fig. 1 and furthermore, that the grouping and treatment after one-and-a-half years did not have a statistically significant effect. The Mann-Whitney U-test was chosen as the most suitable for the comparison of these statistically small samples (Siegel, 1956). No significant difference of the antibody titre could be established at any stage of the experiment when the monthly values of all six animals in the dark were compared with the control animals. The 5 per cent level of significance could only be reached in two months of the actual treatment and only in the difference between the four animals with anti-J in the dark and in the control group.

It may be mentioned here that not the slightest degree of blindness was observed among the animals in the dark stable. Their eyes underwent no change and the animals themselves were always healthy and their growth rate was higher than that of the control animals as can be seen from Table 4.

TABLE 4.—*The live weights of the animals subjected to the light (control) and dark treatments*

	Weight at different stages of the experiment (lb.)		
	Beginning 8 July, 1959	Middle 18 January, 1960	End 18 July, 1960
Light group—			
No. 8203	895	1,254	1,459
8343	818	1,149	1,353
8344	826	1,180	1,385
8388	754	1,028	1,153
8395	781	1,061	1,181
8851	567	801	955
Average	773·5	1,078·8	1,247·7
Dark group—			
No. 8206	785	1,216	1,488
8744	778	1,075	1,228
8788	793	1,164	1,438
8842	758	1,232	1,269
8858	600	925	1,077
8868	681	1,151	1,500
Average	735·8	1,127·2	1,333·3

From Table 4 it can be calculated that the average difference in growth between the light and dark groups was 86·1 lb. for the first half year, 37·2 lb. for the second half year and 123·3 lb. for the total period in favour of the animals in the dark stable.

From a summary of the results it appears that temperature might be the main climatic factor which has an influence on the concentration of normally occurring isoantibodies. If the temperature curves in Fig. 3 are compared, it can be seen that the minimum and maximum temperatures indoors almost throughout the whole year were higher than the respective temperatures outdoors. Table 5 shows these differences and, moreover, the temperatures and the air humidity at the time of reading, i.e. at 8.10 a.m. for both groups.

TABLE 5.—*Comparison of temperature (* °C*) and air humidity* (%) *appertaining to the indoor and outdoor groups*

Month	Max. temp. difference: Indoors minus outdoors	Min. temp. difference: Indoors, minus outdoors	Reading time at 8.10 a.m.			
			Temperature		Air humidity	
			Indoors	Outdoors	Indoors	Outdoors
July.....	2·49	4·77	9·33	8·40	78·2	76·0
August.....	1·81	4·85	10·96	10·85	69·8	63·7
September..	2·44	4·92	17·06	16·17	67·9	61·8
October.....	3·72	4·68	24·36	20·52	64·6	58·7
November..	4·34	5·10	24·80	19·98	68·8	67·9
December...	−1·31	4·64	26·05	21·22	71·4	73·6
January.....	0·31	4·66	26·72	21·65	67·9	67·8
February....	−0·79	4·00	26·23	21·71	76·0	71·4
March.....	3·04	5·68	26·26	20·12	83·2	77·9
April.....	1·85	6·85	19·35	16·52	87·3	78·2
May.....	2·96	7·73	14·26	12·11	86·4	72·5
June.....	0·86	5·22	11·06	8·32	87·6	77·1
July.....	0·08	4·43	11·35	8·17	83·0	79·1

From Table 5 it can be gathered that a difference of 4 to 6 °C existed between the two groups in respect of the minimum temperature and also the temperature taken at 8.10 a.m. The difference is smaller for the maximum temperature, and it is interesting to notice that the actual levels of both temperature and air humidity do not seem to have an influence on the forming of normally occurring isoantibodies. In European and American studies no temperatures are mentioned. The average maximum temperature for instance in Norway would range about 10 °C below the given values in Fig. 3 and Table 5, but the formation and variation of normally occurring isoantibodies could be established, nevertheless (Braend, 1959). Therefore, it is believed that not the temperature level is important but the gradual change in temperature supplies the stimulus for the changes in the antibody concentration.

Another small experiment was performed to establish whether the small difference of about 5 °C between summer and winter temperatures could possibly cause the increase and decrease in the production of normally occurring isoantibodies. Near the equator, e.g. in Kenya, the temperature differences between summer and winter are very small and one would expect that the variation, if any, in the titre of normal

antibodies would be much smaller than in the animals used in the experiment previously discussed. Through the kindness of Dr. Lampkin of the East African Veterinary Research Organization, Muguga, Kenya, it was possible to obtain serum samples of the Boran breed. The first batch of 96 serum samples was tested during January, 1960, but only seven sera contained normally occurring isoantibodies. The low frequency of normal antibodies found here is related to age (all animals were between six to 12 months old) and will be discussed elsewhere. Samples of the seven animals with normal antibodies in their serum were sent to Onderstepoort for titre determinations every three months until January, 1961.

The results are presented in Table 6. The average scores for the antibody level are compared with the average maximum and minimum temperatures as obtained from the East African Agriculture and Forestry Research Organization.

TABLE 6.—*The changes in the level of normally occurring isoantibodies in relation to temperature variations in the equatorial regions*

Muguga, East Africa	January	April	July	October
Av. scores in antibody level (seven animals)	29·8*	27·7	18·6	18·6
Av. Max. Temp., °C (1956–58)†	21·7	21·6	17·5	22·3
Av. Min. Temp., °C (1956–1958).....	11·4	12·8	8·6	11·2

* The scores of all January samples (1960 and 1961) are averaged.
† No later data were available.

The small difference of less than 5 °C between summer and winter temperature (both maximum and minimum) appears to be enough to raise or lower the antibody titre. It could, however, not be established if the variation under such circumstances is as great as under South African conditions.

In addition to the previously mentioned results, viz. that temperature does have the most stimulating effect on the production of normally occurring isoantibodies, and that, due to a time lag, the lowest or highest levels of antibody concentration being reached some two to three months after the lowest or highest levels of temperature, it could also be shown that any gradual change in temperature appears to be effective in bringing about the variation in the level of normally occurring isoantibodies.

D.—Veterinary Aspects of Normally Occurring Isoantibodies

Apart from the influence of normally occurring isoantibodies on blood transfusions, which will be discussed elsewhere, several comparative tests were performed to study a possible interference of normal antibodies on serological tests for the detection of antibodies against bacterial and virus diseases. It has been proved that antibodies in general are very specific (Burnet & Fenner, 1953) and only react with their corresponding antigens, but it was decided to study a possible influence of normal antibodies on serological antibody tests for bacterial or virus antigens, or vice versa, viz.:—

(*a*) the presence of " normal " antibodies might increase or decrease the antibody titre for a disease antigen;

(*b*) the antibodies against antigens derived from disease-producing agents might have an influence on the " normal " antibodies.

Brucellosis.—Several tests have been compared: of 18 animals tested for *Brucella abortus* four gave positive, one suspicious and 13 negative reactions. Tests of the same sera gave seven positive and 11 negative reactions for normally occurring antibodies. Conformity was obtained in ten cases which equals 55·5 per cent.

Brucellosis	*" Normal " antibodies*	*Number*
Pos.	Pos.	1
Susp.	Pos.	1
Neg.	Neg.	8
		10

Tuberculosis.—Here the results were compared in the same way: of 34 animals tested for tuberculosis 17 gave positive and 17 negative serum reactions. Tests of the same sera gave 21 positive and 13 negative reactions for normally occurring antibodies. Conformity was obtained in 18 cases which equals 52·9 per cent.

Tuberculosis	*" Normal " antibodies*	*Number*
Pos.	Pos.	11
Neg.	Neg.	7
		18

Paratyphoid in calves.—In a few cases it was shown that four to eight days old calves had very strong anti-J with a titre of 1/256 and 1/512, which was much higher than the antibody titre of their mothers. The comparison with the paratyphoid tests gave no results.

Lumpy skin disease.—A total of 34 animals was inoculated with lumpy skin vaccine nine months before the comparative tests were performed. Of these 15 showed a demonstrable rise in antibodies after inoculation. Tests of the same sera gave 15 positive and 19 negative reactions for normally occurring antibodies. Conformity was obtained in 16 cases, i.e. 47·1 per cent.

Lumpy skin	*" Normal " antibodies*	*Number*
Antibody rise	Pos.	6
No antibody rise	Neg.	10
		16

Sweating sickness.—Sixty-three Nguni blood samples were received from Swaziland; 32 animals had had severe attacks of sweating sickness and 31 animals were bled as controls for comparison. The tests of these 63 sera gave 27 positive and 36 negative reactions for normally occurring antibodies. Conformity was obtained in 38 cases, i.e. 60·3 per cent.

Sweating sickness	*" Normal " antibodies*	*Number*
Pos.	Pos.	17
Neg.	Neg.	21
		38

Summarising these results no influence of normally occurring isoantibodies on other antibodies or vice versa could be established.

Another interesting question, arising from the seasonal variation of normally occurring antibodies, is: Would it be possible to find a similar variation in the concentration of antibodies against bacterial antigens?

Several factors have to be considered in this connection, such as the age of the infection, body reaction, the interval between infections and antibody response. Will an animal infected, e.g. with *Brucella abortus* show the same concentration of antibodies throughout the period of infection, or will this rise and fall due to seasonal variation?

Van Drimmelen (1953) reported a seasonal exacerbation and recession of serologically positive Brucella agglutination reactions in a big herd of about 750 open range cattle. In this herd routine Brucella tests were performed annually during June from 1930 to 1948. In June, 1949 five reactors were found and testing of the herd at about three monthly intervals in January, April, June and October was instituted. The incidence of reactions showed a seasonal increase which is demonstrated in Fig. 4 where similar results for females and males are given separately.

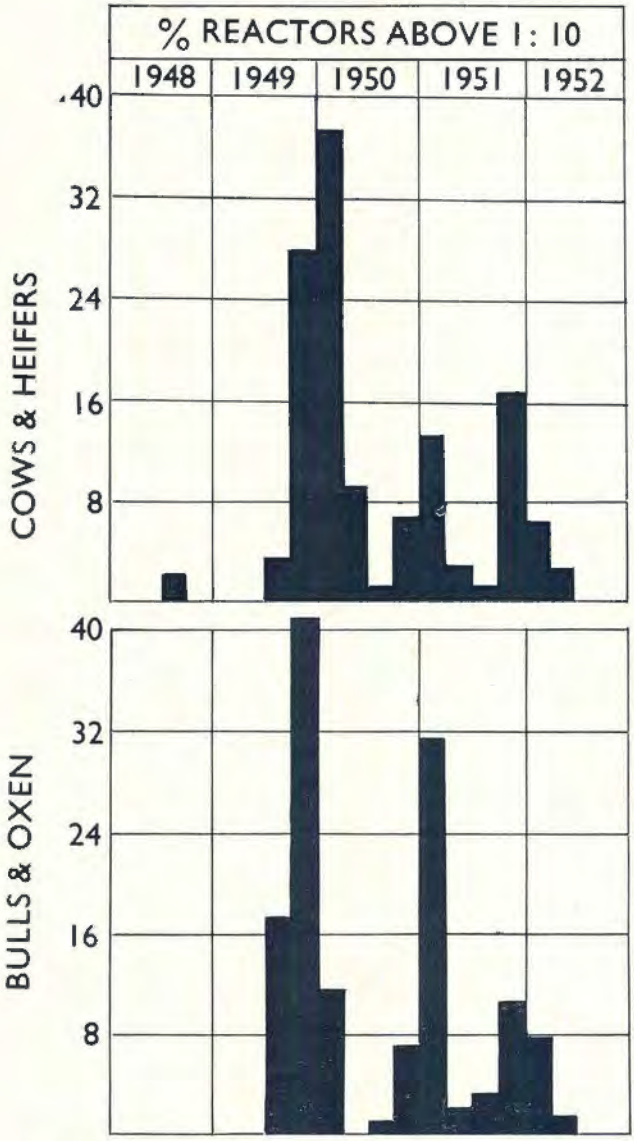
At that stage it was believed that the variation in titres and reactions was connected with the stage of gestation, because the highest titres were encountered during the calving season.

Aebli (1959), in his thesis on *Brucella abortus* in Switzerland, published the titre values on 6,624 animals which were tested only twice. When taking only positive, suspicious and negative reactions into account, 89·6 per cent of all animals remained in the group classified before, 8·5 per cent showed decreased and 1·9 per cent increased reactions.

The author carefully analysed these data and took all titre steps into consideration. Furthermore, the material was divided in two groups: (1) in which the first titre test was performed in spring and the second test in autumn; and (2) in which the first test was performed in autumn and the second test in spring. Table 7 gives the calculations.

TABLE 7.—*Comparison of serologically positive Brucella agglutination titres in two successive tests*

Season when tests were performed	Brucella agglutinin reactions in relation to the first tests			
	Lower	Same	Higher	Total No.
	%	%	%	
Both tests throughout the year.....	28·6	47·9	23·5	6·555
First test in spring.....	18·8	47·1	34·1	845
Second test in autumn.....				
First test in autumn.....	35·2	42·4	22·4	474
Second test in spring.....				



The Seasonal Variation of Serologically Positive Brucella Agglutination Reactions (after V. Drimmelen, 1953)

The differences between the last two test groups: (first test in spring, second test in autumn; and first test in autumn and second test in spring) are highly significant:—

$$\chi^2 = 48{\cdot}5;\ 2\ \text{d.f.};\ P = 0{\cdot}001***.$$

Aebli also suggested that the variation in serologically positive Brucella agglutinin reactions was connected with the stage of gestation, but was unable to prove this suggestion.

Summarising these two findings it may be stated that a definite seasonal variation can occur in the concentration of antibodies against diseases as determined by any serological test. It should be of great value to investigate this problem more fully, not only in the case of *Brucella abortus* but also in other bacterial diseases.

Summary

One type of normally occurring isoantibodies in cattle serum, which has not been described before and which corresponds perfectly with the immune anti-Y_2, has been demonstrated.

A seasonal variation of normally occurring isoantibodies has been established. In all groups of animals, the highest titres were found to be present during the late summer and the lowest titres during the late winter. There appears to be no difference between sexes and among breeds in this seasonal variation, which is not influenced by feeding.

A correlation of climatological factors and naturally occurring isoantibodies was made and it is believed that radiation and temperature may have an influence on the seasonal variation of normal antibodies.

An experiment was carried out to exclude any form of light influence on six animals with normally occurring isoantibodies in their serum. In spite of this the seasonal variation in their antibody level was found to be similar to that in a control group exposed to light. It appears that of the climatic factors temperature has the most stimulating effect on the production of antibodies, but that a retardation of about two to three months takes place in the antibody producing systems. Any gradual change in temperature appears to be effective in bringing about an increase or decrease in the level of normally occurring isoantibodies.

A corresponding variation of antibodies against *Brucella abortus* to that of normally occurring isoantibodies could be demonstrated.

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