Studies of Johne's Disease in Canada. XII. Electrophoretic Analysis of Changes in Serum Proteins in Infected Cattle and Sheep

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ABSTRACT

Electrophoretic examinations were made on sera collected monthly for a period of eleven months from ten cattle naturally or experimentally infected with Johne's bacilli and from ten contact sheep. With one exception, the percentage estimates for the four major classes of serum proteins, albumin, alpha$_1$-, alpha$_2$-, beta- and gamma-globulins did not differ significantly in sera from infected and non-infected, presumably healthy cattle. One cow with a persistently high complement-fixing titre with Johne's bacillus antigen, showed an exceptionally high proportion of gamma-globulin in its serum. The percentage of gamma-globulin tended to be higher in sera of contact sheep than in that of normal sheep sera but the monthly variation in the relative proportion of these and other globulins showed no evident relationship to the fluctuations observed in the specific complement-fixing and anti-complementary properties of these sera.

Introduction

In some infectious diseases definite changes occur in the serum protein pattern, in others very little departure from the normal picture is observed. The disease process may produce an increase in the rate of catabolism of particular classes of proteins or may interfere with their production through temporary or permanent injury to certain organs and tissue systems. The magnitude of the effect depends upon the severity of the disease and the response of the host (1). The most frequently recorded change is an increase in gamma-globulin and a fall in albumin. Where there has been extensive tissue destruction, there may be an increase in alpha-globulins, while in diseases affecting the liver both beta- and gamma-globulins may be high and albumin low. Since serum protein values vary considerably in individual normal animals, examination of a single serum specimen rarely affords conclusive evidence of such changes, unless indeed the picture is conspicuously abnormal. Serial serum samples must be analyzed to determine whether a disease process has had a temporary, protracted or even permanent effect upon the over-all protein metabolism of the animal.

As recorded in paper XI in this series (2), individual differences in the complement-fixing titres with Johne's bacillus antigen have been observed in the small group of Shorthorn cattle with Johne's disease maintained at this Institute. The small number of Suffolk sheep maintained in contact with these infected cattle have also shown variations in complement-fixing properties. Some of the sheep sera have been very anticomplementary, particularly during the summer months, an activity that has introduced considerable difficulty in the interpretation of the diagnostic significance of the reactions recorded with the Johne's bacillus antigen. These differences in the serological behaviour of the

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cattle and sheep, prompted an investigation of their electrophoretic serum patterns with a view to determining whether these reflect differences in the serum protein profile.

Materials and Methods

Blood serum samples were collected monthly from ten cattle and the ten sheep in our Johne's disease herd during the period of January 22 to December 9, 1963. All sera were examined by electrophoretic methods using cellulose acetate.

The three oldest cattle were survivors of the original group purchased as johnin-reactors in 1957 and 1958 from the owner of a herd in which an animal had died of confirmed Johne's disease (2). No. 29008 was a normal bull brought into the herd in May 1963. The other six cattle were 1959, 1962 and 1963 calves of these cows raised in contact with the infected animals. Four of the six as calves were experimentally infected with Johne's bacilli by oral dosing.

Sheep

Four of the ten sheep were obtained from the same farm as the Johne's disease cattle, and at the time of purchase showed a positive reaction in the johnin test. The other six sheep were 1959, 1960 and 1961 lambs of the infected sheep raised on our premises in contact with the infected cattle. Two of them, Nos. 30301 and 40097, were infected experimentally in July and October 1960, respectively.

Electrophoresis Technique

In the electrophoretic examinations, a Shandon Universal Electrophoretic Apparatus with a Volkam power pack was employed with current held constant at 6 mA. In each run six cellulose acetate strips, 2.5 by 12 cm., were placed over an 8 cm. gap. Veronal acetate buffer, pH 8.2, ionic strength 0.05 was used. A period of two hours was allowed for electrophoresis, after which the strips were removed, stained with 0.2 per cent Ponceau S in 3 per cent trichloracetic acid, and decolourized with 5 per cent acetic acid solution. A Spinco Analytrol Model RB was used in scanning the stained strips and in integrating the results.

The individual protein populations represented by the peaks obtained on each graph were identified by the distance each migrated relative to its own albumin band. This facilitated a comparison of the corresponding protein populations shown within a day's test and between tests performed on different days. In each experiment a sample of normal bovine or ovine serum was run in parallel with serum from five infected animals of the same species.

Results

To establish a base line for evaluating the changes in serum proteins in the infected cattle and sheep, sera from a number of apparently healthy animals of each species were analysed by the same electrophoretic procedures. A normal bovine or ovine serum was included in each electrophoretic run with the sera from five infected animals of the same species. Five main peaks were observed for all sera which have been designated I to V. The major protein populations within these five zones, beginning with the most rapidly migrating one, were presumed to represent albumin, alpha$_1$, alpha$_2$, beta- and gamma-globulins. In the electropherograms of some of the sheep sera, a small but well-defined peak was to be seen between zones I and II or as a shoulder on the former peak. It appeared more conspicuous in the tests made in early spring and in October. On repetition of the electrophoretic examination, this subdivision was not always reproduced. This area has been included in Fraction I in estimating percentages.

The electropherograms of several serum samples from the infected cattle and sheep, showed two well defined peaks in region V, one appreciably higher than the other. These were considered to represent gamma$_1$- and gamma$_2$-globulin populations. Other Johne's disease and normal sera exhibited only one peak in region V with a shoulder suggestive of a second protein population. The percentages of the five major protein classes estimated from these curves are given in Tables I and II. Examination of these tables will show values for normal cattle and sheep that correspond relatively well with those reported by other investigators using electrophoretic methods (1, 3-11).

Cattle Sera

Although the percentages of components I to V varied very considerably for individual infected cattle, the mean values for

TABLE 1 Mean serum protein percentages for individual serum samples from normal cattle and for monthly serum samples from ten cattle with Johne's disease; estimated from electrophoretic results

Number of animal	Year of birth	Number of sera tested	% I (albumin)	II (alpha$_1$-globulin)	III (alpha$_2$-globulin)	IV (beta-globulin)	V (gamma-globulin)	Remarks
N. cattle.....		12	35.2 ± 3.63	3.5 ± 0.75	14.1 ± 2.14	15.1 ± 2.34	32.2 ± 3.05	Twelve cattle
N. cattle.....		22	38.6 ± 5.65	3.8 ± 1.49	14.7 ± 2.49	12.5 ± 2.85	30.7 ± 1.53	Three cattle
J. cattle								
40054.....	1955	11	39.4 ± 3.31	3.6 ± 1.29	13.3 ± 2.15	12.4 ± 1.66	31.5 ± 4.45	
40059.....	1956	11	33.5 ± 2.45	3.8 ± 1.69	12.4 ± 1.56	12.4 ± 1.97	38.03 ± 3.60	High C.F. titre throughout
40060.....	1954	11	39.0 ± 3.50	4.1 ± 2.25	14.0 ± 2.55	11.9 ± 1.70	29.6 ± 4.55	Died April, 1964**
29208.....	1962	6	36.3 ± 3.47	3.2 ± 1.00	13.5 ± 1.00	13.5 ± 1.37	33.7 ± 2.48	Introduced into herd in 1963.
29400.....	1963	6	40.2 ± 2.87	3.6 ± 1.40	14.8 ± 3.30	13.6 ± 1.01	27.8 ± 5.94	
29399.....	1963	9	42.8 ± 1.84	5.0 ± 1.70	13.9 ± 1.88	12.5 ± 1.60	25.8 ± 2.40	High C.F. titre in December
24703*.....	1959	11	38.5 ± 2.81	4.8 ± 1.96	12.8 ± 2.74	11.8 ± 2.79	32.1 ± 3.62	
24841*.....	1962	9	36.2 ± 4.71	5.9 ± 2.07	15.1 ± 4.07	12.7 ± 2.80	29.9 ± 5.22	Died September 1963** High C.F. titre
29083*.....	1962	11	37.3 ± 5.48	5.7 ± 3.77	13.0 ± 2.01	11.4 ± 2.17	32.6 ± 6.72	
29084*.....	1962	11	38.6 ± 6.01	5.9 ± 3.08	12.8 ± 3.92	11.9 ± 3.29	30.4 ± 5.94	

*Experimentally infected with Johne's bacilli. February 1960 (No. 24703), March 1962 (No. 24841) and June 1962 (No. 29083 and No. 29084).
**Died of confirmed Johne's disease.

TABLE II Mean serum protein percentages for individual serum samples from 10 normal adult sheep and for monthly samples from 10 sheep in contact with cattle with Johne's disease; estimated from electrophoretic results

Number of sheep	Year of birth	Number of sera tested	% I (albumin)	II (alpha$_1$-globulin)	III (alpha$_2$-globulin)	IV (beta-globulin)	V (gamma-globulin)	Remarks
N. Sheep.....		10	46.4 ± 3.94	7.2 ± 2.26	11.3 ± 2.33	9.7 ± 2.94	23.2 ± 4.75	Individual sheep
40104.....	1956	11	37.7 ± 6.30	6.8 ± 1.14	9.4 ± 2.02	9.4 ± 1.76	36.4 ± 6.09	
40105.....	1956	11	32.0 ± 6.30	8.3 ± 1.82	9.8 ± 1.33	9.2 ± 3.10	40.8 ± 4.00	
32028.....	1956	11	39.8 ± 6.54	5.6 ± 1.95	9.6 ± 1.46	10.8 ± 3.41	34.2 ± 3.93	
24976.....	1960	11	35.3 ± 8.15	6.6 ± 1.42	10.3 ± 2.25	9.4 ± 2.51	38.4 ± 6.94	
24728.....	1960	10	37.0 ± 4.81	6.4 ± 1.09	10.3 ± 1.05	10.0 ± 2.98	36.4 ± 0.64	
29362.....	1961	11	39.5 ± 5.97	7.2 ± 1.32	10.1 ± 2.95	9.3 ± 2.23	33.9 ± 5.22	
24767.....	1961	11	36.1 ± 5.67	7.4 ± 1.93	9.3 ± 1.98	10.1 ± 2.65	28.0 ± 4.31	
24775.....	1961	10	37.8 ± 5.87	7.8 ± 1.50	10.1 ± 2.39	9.6 ± 2.08	34.8 ± 4.60	
40097*.....	?	11	36.0 ± 6.68	6.7 ± 1.41	9.0 ± 1.76	8.4 ± 1.76	39.8 ± 4.29	
30301*	1959	11	41.5 ± 6.19	7.8 ± 1.44	9.4 ± 1.24	9.0 ± 2.43	32.4 ± 5.15	

*Experimentally infected with Johne's bacilli, July and October 1960 respectivelv.

the ten cattle in the Johne's disease herd agreed quite closely with those of the normal controls. The separation into two peaks in region I was more conspicuous with sera from Nos. 40060, 29084 and 29400. The highest mean percentage for gamma-globulin and the lowest for albumin were recorded for No. 40059, the older cow with the persistently high complement-fixing titer. The two other older cows, Nos. 40054 and 40060, which had shown little reaction in complement-fixation tests, had gamma-globulin values relatively comparable to those of the normal cattle. As would be anticipated, the proportion of gamma-globulin in the serum of both of the 1963 calves, Nos. 29400 and 29399, was lower than in the serum of the adult cattle.

Some variation in the range and mean percentages for the main serum components was observed in the monthly tests. For example, in January the gamma-globulin values varied widely, the mean being lower than in any other month whereas alpha$_1$-globulin values were highest. During the last three months of the year, the proportion of alpha$_1$-globulin appeared to fall in the serum of all cattle. In six of the cattle, the percentages of beta-globulin were lowest in March whereas in two others they were slightly elevated. There was less monthly variation in albumin and alpha$_2$-globulin percentages. In addition to the technical variation inherent in these electrophoretic assays of serum proteins, the possible influence of nutritional and other seasonal factors should be taken into consideration in evaluating the importance of variations in monthly percentages of the different serum protein components.

Sheep Sera

The mean monthly values in albumin were definitely lower for the ten contact sheep than for the ten normal sheep, but the mean percentages of gamma-globulin were considerably higher. They were highest for No. 40105, one of the older sheep, and No. 40097 one of the experimentally-infected animals. The mean percentages of albumin, alpha- and beta-globulins did not differ significantly in individual sheep.

In the sheep sera as with the cattle sera, certain monthly differences were noted in the range and mean percentages of the different serum components. For example, the June and early July samples from the contact sheep had the lowest relative albumin concentration whereas those of January and November were the highest. Furthermore in these particular months the proportion of gamma-globulin appeared especially high. No monthly trend was noted in relation to the alpha-globulins. To determine how much the variation between values for monthly samples depended upon their being tested at different times, ten of the monthly bleedings from No. 40097 were examined together in two different electrophoretic runs. The mean percentages estimated for the five protein groups (34.1, 7.1, 3.2, 9.5 and 41.1 per cent) agreed relatively well with the mean values for tests made monthly.

Discussion

When the results of the protein analysis of the cattle and sheep sera were compared with those of the complement-fixation tests for the presence of antibody, some possible relationship between the latter and the level of gamma-globulin was suggested in the case of certain individual samples. Among the cattle, No. 40059, as mentioned earlier, had the highest mean percentage of gamma-globulin in its serum and had a persistently high complement-fixing titre. In December a sharp rise in titre, from less than 5 to 100, was recorded in No. 29399 which was accompanied by a slight increase in the percentage of gamma-globulin: from 26.8 in November to 28.5 per cent in December. However, in the serum of No. 29400 which exhibited no rise in complement-fixing properties, some increase in gamma-globulin was also apparent.

Similarly in the case of the sheep, no consistent correlation between the antibody titre and serum protein profile was evident. Possibly coincidentally, during February and March, when most of the sheep appeared negative in complement-fixation tests, the mean percentage values for gamma-globulin tended to be low. No. 40105, which displayed some degree of complement-fixing-activity throughout the period of observation had a higher proportion of gamma-globulin in its serum than most of the other sheep. The last three serum samples from this sheep were so anticomplementary, however, that the significance of the complement-fixation reaction with antigen was difficult to evaluate. No. 40097, also with a higher than average percentage of gamma-globulin, showed

marked anticomplementary properties in six of eleven serum samples. Only the first and last samples from No. 24767, the sheep with the lowest mean gamma-globulin value, reacted appreciably in the complement-fixation test; the other nine samples were negative. In No. 29362 with titres of 5 to 10 during the last five months of testing, no rise in gamma-globulin was recorded during the same period. No. 30301, with a low mean serum gamma-globulin percentage also showed some complement-fixing activity in later serum samples.

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Utero-Ovarian Disorders Associated with use of Medroxyprogesterone in dogs

Medroxyprogesterone acetate, a derivative of progesterone has exceptionally high and greatly prolonged progestational activity. A single injection has successfully delayed estrus for 6 months or longer followed by a return to a normal estrus cycle. Recently, undesirable effects on reproductive tissues and general health have been observed in dogs previously given injections of this drug for the prevention or alteration of estrus. The authors report on the clinical and pathological changes observed in 5 dogs given the drug and subsequently submitted for treatment to the Angell Memorial Hospital. Four were less than 2 years old. The most common sign at the time of examination was vaginal discharge. Laboratory findings were not consistent but a radiograph usually helped to confirm the clinical diagnosis of an enlarged, fluid-filled uterus. Pathological lesions were similar and unusual. Uteri were enlarged, rather ballooned. None were severely congested. Exudate was similar in all cases; thick, translucent, non odorous, and extremely tenacious. Microscopic lesions were essentially a cystic endometrial hyperplasia, characterized by an unusual amount of secretion. Large numbers of atretic follicles and sclerotic remnants of ova or zona pellucidae as well as perifollicular haemorrhage were seen in the ovaries. These clinical and pathological changes are mimicked to some degree by changes seen during pseudocyesis. The authors conclude by stating that due to the limited number of cases, absence of controls and lack of data on utero-ovarian disorders it is impossible to draw firm conclusions.

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The Anatomy of the Horse — A Pictorial Approach, by Robert F. Way, V.M.D., M.S., and Donald G. Lee, V.M.D. 214 pages, 93 plates. J. B. Lippincott Company, Montreal. Price $13.50.

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