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THE FIBRINOGEN CONCENTRATION IN BLOOD OF DAIRY COWS AND ITS INFLUENCE ON THE INTERPRETATION OF THE GLUTARALDEHYDE AND FORMOL-GEL TEST REACTIONS*

By

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LIBERG, PER: *The fibrinogen concentration in blood of dairy cows and its influence on the interpretation of the glutaraldehyde and formol-gel test reactions.* Acta vet. scand. 1978, *19*, 413—431. — It has earlier been shown that the formol-gel test on serum and glutaraldehyde test on whole blood are simple and rapid methods for evaluation of the immunoglobulin status in the cow. Both tests function as coagulation tests in which aldehyde groups cross-link basic blood globulins at their NH_2-groups, forming polymerisates. The glutaraldehyde has in whole blood the capacity to polymerize not only immunoglobulins but also fibrinogen.

This investigation was made in order to study whether the fibrinogen level may influence the result of the glutaraldehyde test, so revealing any differences between the results of that and the formol-gel test carried out on serum. In 92 cows with a variety of clinical disorders (most of them with inflammatory processes) the total protein, albumin, total globulin concentration and albumin/globulin ratio in serum and fibrinogen concentration in plasma were recorded. The material was grouped according to glutaraldehyde and formol-gel test reactions.

It is shown that increases in the fibrinogen level have an effect on the results of the glutaraldehyde test. A positive glutaraldehyde test in more acute processes is ascribed to a heavy rise of plasma fibrinogen in its capacity of acute-phase protein. A positive glutaraldehyde test in chronic diseases may be viewed as a result of interaction between high immunoglobulin concentrations and elevated fibrinogen concentration.

In conclusion the fibrinogen and immunoglobulin status of blood is important to assess in many diseases of cattle. The semiquantitative tests described for field use can separately, or especially in parallel use, provide valuable information about the character and development of a disease and may be regarded as good substitutes for the sedimentation rate (SR), which is not demonstrable in cattle.

bovine fibrinogen; bovine serum proteins; formol-gel reaction; glutaraldehyde test; acute and chronic inflammations.

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The erythrocyte sedimentation rate (ESR) is so slow in cattle that it is not a usable diagnostic aid in this species. Since one of the main reasons for rapid ESR is an increase of the plasma fibrinogen concentration, the determination of this concentration in cattle would to a large extent have the same diagnostic and prognostic value as the determination of ESR in other species (*McSherry et al.* 1970, *Ek* 1972).

Fibrinogen belongs to the group of "reactive" proteins. The fibrinogen level in the blood is elevated in many diseases (e.g. *Schalm* 1970). Large increase is usually associated with inflammation and tissue injury (*McSherry et al.*). Low levels have been observed in liver diseases and "terminal states". A low plasma fibrinogen concentration in a diseased animal has been regarded as a prognostically bad sign.

Fibrinogen is an acute-phase protein. An initial rise of the plasma level is seen within 24 hrs. after a tissue injury (*Laurell et al.* 1976). The increase of fibrinogen in acute infectious diseases reflects the total effect of the process during a certain period prior to sampling, but not the intensity of the process (*Belfrage* 1963). An infection or other injury that is active only during a day or so, therefore, causes only a fairly slight rise of fibrinogen, even if the process is very intensive. An activity of at least three-four days' duration is necessary to cause large changes. After the regression of the acute process, elevated levels of acute-phase reactants remain for one-two weeks.

Even if rises of fibrinogen and other acute-phase reactants are sensitive indicators of acute inflammation, a rise of fibrinogen occurs also in the response of chronic inflammations (*Laurell et al.*). The most important plasma factor, next to fibrinogen, for ESR is the immunoglobulins. A rise of the latter reflects the lymphoid activity and thus, to a large extent, the chronicity of antigen influence (e.g. *Belfrage*).

Relatively few studies of fibrinogen in cattle have been published. Different methods of determination have been used. Fibrinogen values based on thrombin-coagulable protein in plasma are considered most specific and correct (*Jacobsson* 1955, *Grannis* 1970). Values measured in healthy cows have ranged between 2 and 7 g/l, usually with a mean of 5—6 g/l (*Stormorken* 1957, *McSherry et al., Ek, Thomson et al.* 1974).

Fibrinogen determination is valuable for detection of inflammatory and traumatic states in cattle. A rise of fibrinogen ap-

pears to be a more sensitive indicator of an inflammatory state than the total number of leukocytes in blood (*Schalm* 1970, *Sutton & Hobman* 1975).

The formol-gel and glutaraldehyde tests are simple semi-quantitative methods for detection of hyperimmunoglobulinaemia in cattle (*Liberg* 1973, *Sandholm* 1974). They are coagulation tests in which aldehyde groups cross-link basic blood globulins at their NH_2 groups, so forming polymerisates.

The formol-gel test is performed on serum. The rise of total globulin in connection with positive formol-gel reactions is due almost exclusively to a rise of immunoglobulins. The glutaraldehyde test is done on whole blood. In this test the glutaraldehyde has been found to polymerize not only immunoglobulins but also fibrinogen (*Sandholm*). Any discrepancies between the results of the two tests may be due to the fact that they do not measure exactly the same parameters (*Liberg et al.* 1975).

The present investigation was made in order to study the fibrinogen concentration in cows with different pathological processes of a primarily inflammatory character and to assess the influence of the fibrinogen concentration on the result of the glutaraldehyde test and on any differences between the results of the glutaraldehyde and formol-gel tests.

MATERIAL AND METHODS

The material consisted of 30 clinically healthy cows and 92 cows with a variety of clinical disorders. The most common diagnoses were traumatic peritonitis (29 cows) and abomasal displacement (10). Among the other diagnoses were mastitis, endometritis, polyarthritis/laminitis, endocarditis, urinary tract disorders, abscesses, poisonings and traumatic muscle injuries. In some cases the diagnoses were unclear. Animals emergency slaughtered were subjected to necropsy. Most of the cows with chronic traumatic peritonitis were necropsied.

The method used for determination of fibrin(ogen) in fresh EDTA plasma was a modification of that devised by *Jacobsson* (1955). The plasma sample was clotted with thrombin* (30 NIH to 1 ml plasma) and the clot was dissolved in boiling 1 N-NaOH. The optical density of the fibrin solution was determined in a Beckman Acta C III spectrophotometer at 280 mμ. The nitrogen

* Topostasin®, Hoffman - La Roche, Basel, Switzerland.

content was determined according to Kjeldahl in the same fibrin solutions which had been used for determinations of optical density. The factor for the conversion of the optical density to g/l fibrin(ogen) was calculated to be 4.7 (s 0.1).

All fibrinogen determinations were carried out in duplicate. The analytical variation (=Sa) was calculated according to the formula $Sa = \sqrt{\frac{\Sigma d^2}{2n}}$, where d is the difference between two single determinations on the same sample and n is the number of duplicate determinations. The relative standard deviation was for clinically healthy cows 1.5 % and for diseased cows 3.8 %.

The total protein concentration in serum was determined by the biuret method, and the albumin spectrophotometrically with bromocresol green (*Doumas et al.* 1971). The globulin content and albumin/globulin (A/G) ratio were calculated from the total protein and albumin values.

The formol-gel test (FR) in fresh serum and the glutaraldehyde test* (GLA) in fresh whole blood were carried out according to *Liberg et al.* (1975).

The packed cell volume (PCV) was determined with a microhaematocrit centrifuge, and leukocytes (WBC) in a Celloscope particle counter.

The results were analysed statistically by Student's t-test.

RESULTS

On the basis of the results of the glutaraldehyde and formol-gel tests the diseased animals were divided into four groups: I = both tests negative; II = FR positive, GLA negative; III = GLA positive, FR negative; IV = both tests positive.

The results are presented in Tables 1—3. All cases of abomasal displacement had negative GLA and most cases also negative FR. Most acute-subacute traumatic peritonites had positive GLA; barley half had also positive R. With one exception the chronic traumatic peritonites were GLA-positive and, likewise with one exception, FR-positive. The results of the tests on cows with the other diagnoses varied greatly (Table 1).

Table 2 shows the protein values of the diseased cows without regard to diagnoses within the various groups in comparison with healthy cows. Cows with negative GLA and negative FR (Group I) had a moderate, significant rise of fibrinogen. The

* Clavu-test®. Orion Diagnostica, Helsinki, Finland.

Table 1. The number of various diagnoses, grouped according to the GLA and FR test reactions (+/—).

Diagnosis	Group:	I	II	III	IV
	GLA:	—	—	+	+
	FR:	—	+	—	+
Abomasal displacement		7	3	0	0
Acute-subacute traumatic peritonitis		4	0	5	6
Chronic traumatic peritonitis		0	1	1	12
Other diagnoses		19	5	7	22
Total		30	9	13	40

globulin value was normal, the albumin and total protein values and the A/G ratio significantly lowered. Cows with negative GLA but positive FR (Group II) also had a moderate rise of fibrinogen, but in addition a significant, moderate rise of globulin. The total protein value was in the normal range, whereas the albumin value and the A/G ratio were significantly lowered. Cows with positive GLA but negative FR (Group III) exhibited a strong rise

Table 2. Fibrinogen in plasma, total protein, albumin, globulin (g/l) and A/G ratio in serum in healthy cows and in diseased cows grouped according to the GLA-FR test reactions (+/—).

		Healthy cows	Diseased cows			
Group			I	II	III	IV
GLA		—	—	—	+	+
FR		—	—	+	—	+
Number		30	30	9	13	40
Fibrinogen	$\bar{x}$	4.0^{a}	5.7^{b}	5.7^{b}	9.6^{c}	9.6^{c}
	s	0.7	1.6	1.6	2.7	0.7
Total protein	$\bar{x}$	76.4^{a}	72.3^{b}	$79.2^{a,c}$	66.5^{d}	86.1^{e}
	s	4.2	6.3	6.0	10.2	8.6
Albumin	$\bar{x}$	42.9^{a}	37.9^{b}	$36.4^{b,c}$	33.3^{c}	$35.4^{b,c}$
	s	2.7	5.3	5.1	7.5	5.7
Globulin	$\bar{x}$	33.7^{a}	34.4^{a}	42.8^{b}	33.5^{a}	50.6^{c}
	s	3.9	4.9	4.6	4.5	10.2
A/G ratio	$\bar{x}$	1.29^{a}	1.10^{b}	0.86^{c}	1.01^{b}	0.74^{c}
	s	0.20	0.27	0.17	0.23	0.23

Values with different alphabetical codes differ significantly ($P < 0.05$).

of fibrinogen, whereas their globulin concentration was normal. Their total protein and albumin values and A/G ratio were significantly lowered. Cows with positive GLA and positive FR (Group IV) exhibited a large rise both of fibrinogen and globulin. Their total protein concentration was elevated, but their albumin value and A/G ratio significantly lowered.

Table 3. Packed cell volume (PCV, l/l) and leukocytes (WBC, $x10^9$/l) in healthy cows and in diseased cows grouped according to the GLA and FR test reactions (+/—).

		Healthy cows	Diseased cows			
Group			I	II	III	IV
GLA		—	—	—	+	+
FR		—	—	+	—	+
Number		30	24	8	13	25
PVC	$\bar{x}$	0.36^a	0.37^a	0.40^b	0.33^c	0.33^c
	s	0.03	0.05	0.05	0.04	0.05
WBC	$\bar{x}$	7.6^a	7.6^a	6.3^a	7.5^a	8.2^a
	s	1.9	3.2	2.1	2.8	2.7

Values with different alphabetical codes differ significantly ($P < 0.05$).

Table 3 shows that the packed cell volume for Group I was normal, whereas in Group II there was a slight, significant rise and in Groups III and IV a slight, significant lowering of PCV. There was no difference in total number of leukocytes either between healthy and diseased cows or between the various groups of diseased cows.

DISCUSSION

Many diseases of cattle which are difficult to prognose are associated with inflammatory states. The processes can generally be surveyed through laboratory tests of different blood proteins. For practical purposes it often suffices to determine the fibrinogen and immunoglobulin levels, since these reveal sufficiently much about the duration, activity and spread of the inflammatory process. The diagnostic and prognostic possibilities are thereby markedly improved.

The present study confirms earlier findings (*Liberg* 1973) that, when the (immuno)globulin concentration is normal, the

formol-gel test will be negative (Table 2, Groups I and III). It also confirms the earlier assumption (*Liberg et al.* 1975) that the discrepancy between the FR results in serum and the GLA results in whole blood appears primarily to be due to the fact that the tests do not measure exactly the same parameters. From Table 2 it is apparent, accordingly, that when the (immuno)globulin concentration was normal, GLA was positive only when the fibrinogen concentration was greatly elevated (Group III), whereas a moderate rise of fibrinogen alone did not yield a positive GLA (Group I). Not even a moderate rise of fibrinogen in combination with a moderate rise of (immuno)globulin yielded a positive GLA (Group II). A positive GLA in more acute pathological processes which have not yet resulted in elevated immunoglobulin levels in the blood must therefore be ascribed to a heavy rise of plasma fibrinogen in its capacity of acute-phase protein. A positive GLA in chronic diseases should be viewed as a result of interaction between high immunoglobulin concentrations concurrently with elevated fibrinogen concentration in the blood.

In comparative glutaraldehyde tests on serum and on plasma great differences have often been found in the reactions, whereas there is close conformity between the formol-gel and glutaraldehyde test reactions when both tests are made on serum (unpublished studies). This, too, confirms that GLA, apart from measuring the immunoglobulin concentration, is also very greatly dependent on the fibrinogen concentration. The study confirms earlier observations (e.g. *McSherry et al.* 1970, *Schalm* 1970, 1972, *Ek* 1972) that the fibrinogen concentration in plasma is an especially sensitive indicator of, in particular, inflammatory disease in cattle. As in earlier studies (*McSherry et al., Ek*) very high concentrations — up to about 20 g/l — were found in individual cases with severe inflammatory processes.

To judge whether haemoconcentration might affect the protein concentrations by dehydration, and thus cause false positive GLA-FR reactions, PCV was determined for the majority of the animals. Group II was found to exhibit a slight increase, but not of such extent as to have a decisive influence on the GLA-FR results. But in view of the limited number of individuals, Group II should be judged with caution in this as in other respects. It is remarkable that Group III and IV, which contained the majority of chronic inflammations, showed the lowest mean

values of PCV. The lower PCV values in these groups were probably caused by mild anaemia in several cases, as indicated by parellel determinations of haemoglobin in some of the cows.

For the total number of leukocytes there was no difference in mean value between healthy and diseased cows or between the various groups of diseased cows, which, in conformity with the finding by *Schalm* (1970), indicates that the total leukocyte count in cows seems to be a less sensitive indicator of inflammatory state than changes in plasma protein fractions.

In conclusion it may be pointed out that the fibrinogen and immunoglobulin status of the blood is important to assess in many diseases of cattle. The semiquantitative rapid tests described — GLA and FR — can separately, or especially in parallel use, provide valuable information about the character and development of a disease. Positive as well as negative test reactions can give very valuable information for diagnosis and prognosis. These rapid tests may be regarded as very good substitutes for the sedimentation rate, which is of no value in cattle.

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SAMMANFATTNING

Fibrinogenkoncentrationen i blod hos mjölkkor och dess inverkan på tolkningen av glutaraldehyd- och formol-geltestreaktionerna.

Det har tidigare visats att formol-geltestet på serum och glutaraldehydtestet på helblod är enkla snabbmetoder för bedömning av immunoglobulinstatus hos ko. Båda testerna fungerar som koagulationstester i vilka aldehydgrupper kopplar basiska blodglobuliners NH_2-grupper. Därvid bildas polymerisat. Glutaraldehyden har i helblod förmåga att polymerisera inte bara immunoglobulinerna utan även fibrinogen. Hos 92 kor med olika kliniska sjukdomstillstånd (flertalet av inflammatorisk karaktär) bestämdes totalprotein-, albumin-, totalglobulinkoncentration och A/G-kvoten i serum samt fibrinogenkoncentrationen i plasma. Materialet grupperades efter glutaraldehyd- och formol-geltestutslagen. Av resultaten framgår tydligt att ökningen av fibrinogenhalten har betydelse för utslagen i glutaraldehydtestet. En positiv glutaraldehydtest vid mer akuta processer kan tillskrivas en kraftig ökning av plasmafibrinogen i egenskap av akut-fas-protein. Positiv glutaraldehydtest vid kroniska sjukdomsprocesser bör ses som ett resultat av samverkan mellan höga immunoglobulinkoncentrationer och förhöjd fibrinogenkoncentration i blodet.

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