Acta vet. scand. 1973, *14*, 712—722.

From the Research Station of the Veterinary Institute, Skara, Sweden.

THE FORMOL-GEL REACTION IN CATTLE*

By

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LIBERG, PER: *The formol-gel reaction in cattle.* Acta vet. scand. 1973, *14*, 712—722. — The correlation between the formol-gel reaction and the concentration of different serum proteins was investigated in 289 cattle with chiefly chronic diseases.

A close correlation, though not linear, was found between the quantity of globulin and the time of gelification. A positive formol-gel reaction within 24 hrs. indicated an elevated globulin level in serum. Increasingly marked rises of globulin were observed with shorter gelification times. A negative formol-gel reaction after 24 hrs. showed that the globulin concentration was at normal level.

The results are discussed in the light of the relevant literature.

It is concluded that the formol-gel reaction is of diagnostic and prognostic value for clinical work within bovine medicine.

formol-gel test; formol-gel reaction; serum globulin; chronic inflammations; cattle.

Chronic infections are the most common cause of hyperglobulinaemia. The duration of the illness and the degree of inflammatory reaction are determinative of the amount of increase of globulin. Acute infections usually cause insignificant or no effect on the concentration of serum globulin.

A knowledge of the absolute or relative globulin concentration may have a significance for diagnosis and prognosis.

Accurate determination of different serum protein concentrations takes a long time and calls for refined laboratory resources. For routine use these laboratory methods are altogether too complicated and time-consuming. There are several simple non-specific protein reactions which can be used instead. These have the character of lability reactions, i. e. certain chemical and colloid-chemistry reactions in whole blood, plasma or serum.

* The investigation was supported by the Swedish Council for Forestry and Agricultural Research.

The principle of the lability reactions is that proteins are converted from a soluble state to state of viscosity, flocculation, precipitation or gelification depending on the reagent system employed.

A quick and simple protein lability reaction is the formol-gel reaction. This reaction was discovered by chance through the observation of gelification when formalin was added to certain pathological sera (*Gaté & Papacostas* 1920). Positive formol-gel reactions were found to occur only in sera with elevated globulin concentrations (*Bing* 1937). The formol-gel reaction (FR) can thus be used for confirmation of hyperglobulinaemia. The applicability of the formol-gel reaction as specific diagnosis of disease or as rapid method for estimation of the globulin concentration in serum has been the object of study by several authors both within human and veterinary medicine (e. g. *Bing* 1937, 1940, *Linke & Steckert* 1949, *Wiesner* 1951, *Bendixen* 1954, *Meric* 1959).

Frimmer (1949) made a thorough study of the background to the formol-gel reaction. In his opinion the gelification with formaldehyde is due to cross-linking between the E-lysin groups of the proteins and adjacent peptide groups of protein molecules, the properties of a protein to form formol-gel being determined by its space structure. Low-molecular protein degradation products have only an insignificant effect on the result of the formol-gel reaction. *Frimmer* also showed the possibility of making the reaction more sensitive by addition of lysin.

Bendixen studied the correlation between the formol-gel reaction and changes of individual protein fractions, determined by paper electrophoresis, in serum of cows with chronic infections. He found that the rise of globulin on a rapidly occurring FR was almost exclusively limited to the γ-globulin concentration, while the α- and β-globulins remained practically unchanged in chronic inflammations. *Meric* unlike several other authors, found no clear relationship between FR and different serum protein fractions in 24 cattle. In particular he found the relationship between γ-globulin concentration and gelification time to be non-uniform.

The first investigation of FR in cattle serum was made by *Pigneur* (1928) in the Belgian Congo. He found a positive FR in all cattle. Owing to the conditions in the Congo area, with protozoal infections among others, *Pigneur* considered the for-

mol-gel reaction to be unusable in that area. He also reported a shortening of the gelification time with rising age of the cattle. He found that the feed, stage of pregnancy and lactation had little influence on FR. *Bouvier* (1936) found FR to be positive in 50 % of all clinically healthy cattle under tropical conditions and therefore unusable for diagnosis in such areas.

Wiesner (1951) studied FR in 27 cows and 72 calves in Germany, all clinically healthy. Of the cows 33 % reacted positively, and of the calves 10 %. *Sikora* (1952) investigated normal slaughter animals, also in Germany. Of 68 calves 98 % showed a negative FR, of 32 healthy young cattle 94 % and of 36 adult cattle 92 %. There was thus a tendency to an increase of the number of positive FR in healthy cattle with rising age. *Bendixen* showed that in healthy cows, especially with rising age, FR often proved to be positive, contrary to the condition in man. He explained this through the fact that cattle normally have a higher globulin level, probably because more often they have chronic inflammation processes of infectious or parasitic origin.

In an investigation of 256 clinically healthy cattle with negative tuberculin test and negative Bang titres 75 % had a negative FR, 17 % faintly positive and 8 % strongly positive (*Wiesner* 1957). *Wiesner* established that the serum protein spectrum in cattle serum strongly deviates from that in man and that therefore serum lability tests have a considerably different outcome. In a statistical investigation of 800 cattle he found no clear correlation between clinical symptoms (chronic infectious diseases) and serum protein composition.

Among 123 clinically and pathoanatomically healthy slaughter cattle subdivided according to age, *Meric* found FR to be negative in all calves below 3 months of age, negative in 92 % of 1- to 2-year-olds, 86 % of 2- to 3-year-olds, and 67 % of 3- to 8-year-olds.

According to *Meric* the formol-gel reaction in cattle above 1 year of age is always positive, if the test is made in plasma, possibly because of the normally high fibrinogen level in plasma of cattle.

FR, accordingly, would seem to be of no value for diagnosis of disease in cattle under tropical conditions. Opinions concerning its value within other geographical areas differ fairly considerably, for which reason an examination of FR under Swedish conditions was considered to be of value.

In the present investigation, therefore, a study has been made of the general value of the formol-gel reaction as rapid method for estimation of the serum globulin concentration and of its value for diagnosis of chronic diseases.

MATERIAL AND METHODS

Blood samples were collected from 289 clinically diseased cattle with mainly chronically inflammatory processes. The material consisted of adult cows, chiefly of Swedish red-and-white breed. Clinical diagnoses were established in 272 cases, in 80 cases confirmed by necropsy, in 44 by bacteriological mastitis-control, in 12 cases by operation.

A normal material was collected from 91 clinically healthy cows, of which 48 were subjected to bacteriological mastitis-control, which revealed mastitis-free udders in all cases.

Total protein was determined by the biuret method.

Albumin was determined spectrophotometrically with brom-cresol green (*Doumas et al.* 1971). The method was standardized against bovine albumin (Miles Lab. Inc.: Pentex® Crystallized Bovine Albumin) the concentration of which was determined by Kjeldahl analysis.

Globulin concentration and A/G ratio were calculated on the basis of total protein and albumin values.

The *formol-gel test* was carried out as follows: To 1 ml serum in a narrow test tube of about 4 ml volume were added 3 drops (0.1 ml) of neutral 35 % formalin solution. Formalin and serum were mixed and the test tube was kept at room temperature. The start time was noted and the test tube was turned upside down at regular intervals during an observation time of 24 hrs. The criterion of positive reaction was that gelification had occurred so that, on reversal of the test tube, the serum remained at the bottom. A negative reaction was considered to exist when the serum was still fluid after 24 hrs.

RESULTS AND DISCUSSION

Fig. 1 shows that there was a close relationship but no direct proportionality between gelification time and serum globulin concentration. The points are gathered around a curve which falls steeply during the first 2 hrs. and then slowly levels off towards the 24-hr. limit. A statistical comparison between the

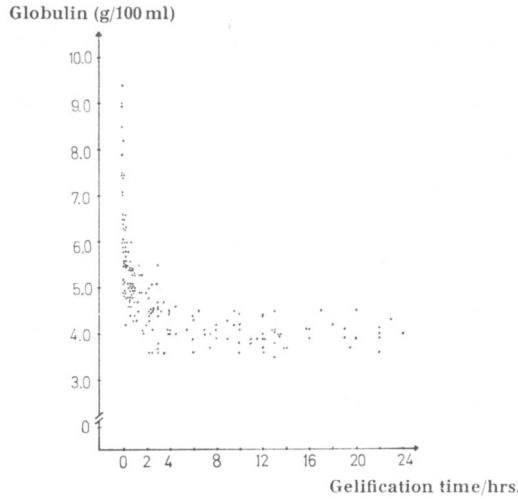

Figure 1. The globulin concentration in serum in relation to gelification time in FR.

mean values of globulin concentration in serum within different time intervals showed significant differences between all groups except between the group with negative formol-gel reaction after 24 hrs. and the normal group (Fig. 2).

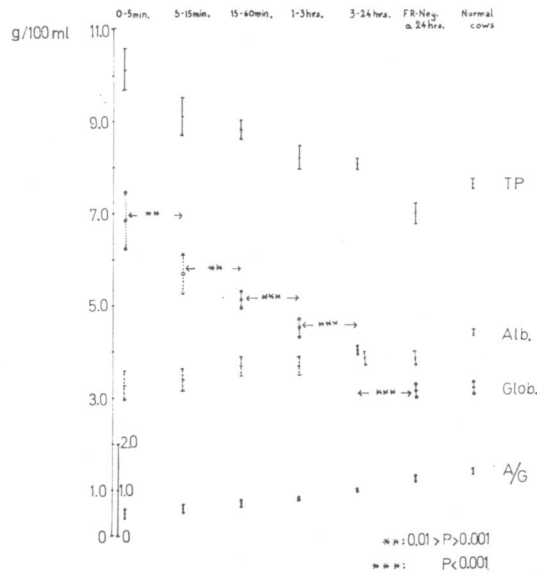

Figure 2. Concentrations of total protein, albumin and globulin and A/G ratio in serum within different gelification time intervals $\bar{x} \pm 95\ \%$ confidence interval.

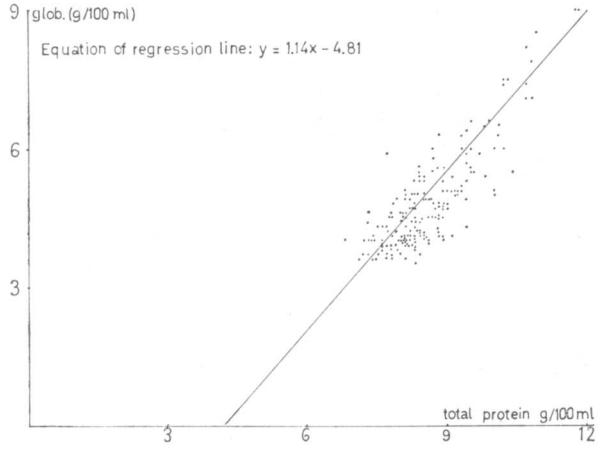

Figure 3. Correlation between globulin and total protein concentrations in serum for samples which are formol-gel positive within 24 hrs.

At elevated globulin level there was a lowered albumin concentration, a lowered A/G ratio and an increased total protein concentration (Fig. 2).

A strongly positive correlation was found between the globulin and total protein concentrations in cows with positive FR within 5 hrs. (Fig. 3).

From repeated protein determinations in a few cases with hyperglobulinaemia, it was noticed that the globulin concentration fell despite progression of the disease.

Practically applicable evaluation criteria are presented in

Table 1. Concentrations of globulin and total protein in serum within different gelification time intervals and for normal cows.

Gelification time	n	Globulin			Total protein		
		$\bar{x}$	95 % conf. int.	approx. value	$\bar{x}$	95 % conf. int.	approx. value
0— 5 min.	22	6.9	0.6	> 6.3	10.1	0.5	> 9.6
5—15 min.	16	5.7	0.4	abt. 5.5	9.1	0.4	abt. 9.0
15—60 min.	38	5.1	0.2	abt. 5.0	8.8	0.2	abt. 9.0
1— 3 hrs.	32	4.5	0.2	abt. 4.5	8.2	0.3	abt. 8.0
3—24 hrs.	67	4.0	0.1	abt. 4.0	8.1	0.1	abt. 8.0
Formol-gel negative cows	98	3.2	0.1	< 3.3	7.0	0.2	< 7.3
Normal cows	91	3.2	0.1	abt. 3.0	7.7	0.1	abt. 7.6

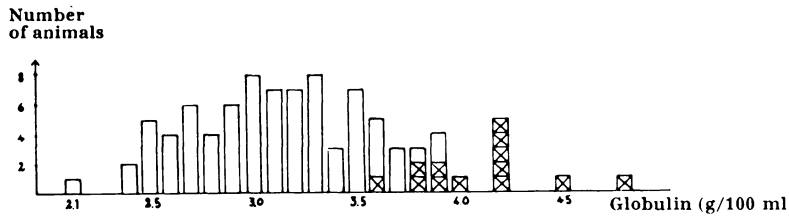

Figure 4. Distribution of globulin concentration in serum by number of animals in the normal material (cross indicates FR-pos.).

Table 1. A positive FR within 5 min. indicates a globulin concentration of above 6 g/100 ml serum. Gelification within 1 hr. implies a serum globulin concentration exceeding 5 g/100 ml, and a positive reaction within 1—3 hrs. a concentration of between 4—5 g/100 ml. A positive formol-gel reaction within 3—24 hrs. indicates a globulin concentration of around 4 g/100 ml. A negative formol-gel reaction within 24 hrs. implies that the globulin concentration in serum does not differ significantly from normal, i. e. about 3 g/100 ml. In the present study the reaction at lower serum globulin concentration than 3.5 g/100 ml was always negative, so that the threshold for a positive formol-gel reaction within 24 hrs. may be assumed to be at this level.

Thirteen of the 91 normal animals (14 %) were formol-gel-positive, most of them with a long gelification time. Only one of them was positive within 3 hrs. In normal animals it may be reckoned that, at least within 3 hrs., the formol-gel reaction is practically always negative, but that in the great majority of entirely healthy individuals it is negative also after 24 hrs. From Fig. 4 it will be seen that the formol-gel-positive reactions within the normal material also correspond to the highest globulin concentrations.

Fig. 5 shows that in several chronically inflammatory processes such as traumatic peritonitis, so-called summer mastitis and pyelonephritis the serum globulin concentrations are generally moderately to heavily elevated, whereas chronic mastitis — other than summer mastitis — often does not give rise to major rises of globulin. Diseases of non-infectious character, on the other hand, are usually accompanied by little or no rise of globulin. Leukosis is accompanied by hypoproteinaemia, hypoglobulinaemia and hypoalbuminaemia.

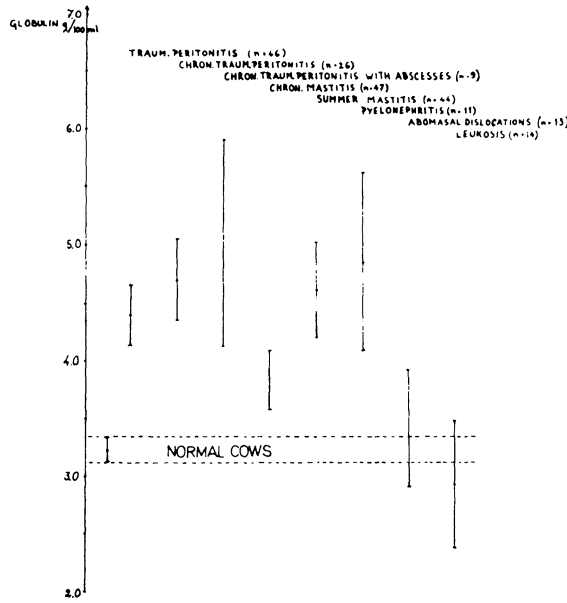

Figure 5. Globulin concentration in serum $\bar{x} \pm 95\,\%$ confidence interval for different diseased states.

The necropsies confirmed that age, degree and extent of the inflammatory process are roughly correlated to the increase of serum globulin, and thus to the result of the FR. Pyogenic reactions and abscesses in different organs or systems of organs gave rise to more marked hyperglobulinaemia than in inflammatory processes without these reactions.

The formol-gel test has been carried out in the same way as in most earlier reported studies, so that the results may be considered to be comparable even if the criteria for positive and negative reactions have differed to some extent. The distinct limit between solid and non-solid state here used for evaluating positive reactions must be regarded as an advantage when employing the test in practice.

Bendixen (1954), in contrast to *Meric* (1959), found the increase of globulin in conjunction with positive formol-gel reactions to be due almost exclusively to the increase of the γ-globulin fraction, which is clearly confirmed by own unpublished observations on agarose gel electrophoretic protein separation.

Observations that positive formol-gel reactions are seen in

clinically and pathoanatomically healthy cattle presumedly can be explained only partly by the fact that older animals "normally" exhibit an elevated globulin level. They may equally well indicate the difficulties encountered in the practical clinical diagnosis of a number of chronic inflammatory processes in cattle, e. g. chronic mastitis. From the results of the present study, however, it is clear that most healthy animals exhibit a negative formol-gel reaction. In contrast to the results found in tropical conditions (*Pigneur* 1928, *Bouvier* 1936 etc.), accordingly, the test has proved usable for diagnosis of disease in adult cattle under Swedish conditions.

Young calves usually show lower serum globulin concentrations than adult cattle. It is probable, therefore, that the globulin concentration in moderate inflammatory processes in calves does not reach the limit at which a positive formol-gel reaction occurs in adults. The lowering of albumin seen in conjuction with a rise of the globulin level can be explained, according to *Bendixen,* by the fact that the higher-molecular globulins substitute the maintenance of normal colloid-osmotic pressure. Hyperglobulinaemia is sometimes seen without any change of the A/G ratio, as in haemoconcentration owing to dehydration. Correlation of the serum protein values to the haematocrit must therefore be considered important for the evaluation of hyperproteinaemia and hyperglobulinaemia.

The globulin concentration decreases in some cases despite progression of the disease. This may be due to the fact that an impaired general condition causes loss of appetite and that the animal therefore does not consume the necessary nutrients for protein synthesis. In cases of reduction of the absolute globulin concentration in the present study a relative increase of globulin has often been observed, while the albumin fraction has decreased even more than the globulin. In general, however, repeated determinations of the formol-gel reaction in protracted disease processes provide means for evaluating whether the inflammatory process is progressing, stagnating or receding.

Acute inflammatory processes do not allow time for an increase of globulin, so that the formol-gel reaction is then negative. The degree and extent of inflammation is correlated to the increase of serum globulin. Determination of the globulin level by means of the formol-gel reaction may therefore be of value for determination of the stage of disease and of its prognosis.

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SAMMANFATTNING

Formolgel-reaktionen hos nöt.

Korrelationen mellan formolgelreaktionen och koncentrationen av olika serumproteiner undersöktes hos 289 nötkreatur med huvudsakligen kroniska sjukdomar.

Ett nära samband, dock icke linjärt, konstaterades mellan globulinmängden och gelbildningstiden. Positiv formolgelreaktion inom 24 timmar angav en förhöjd globulinnivå i serum. Allt mer markerade globulinstegringar iakttogs med kortare stelningstider. Negativ formolgelreaktion efter 24 timmar visade att globulinkoncentrationen låg på normal nivå.

Undersökningsresultaten diskuteras i ljuset av relevant litteratur.

Som konklusion framhålles att formolgelreaktionen har diagnostiskt och prognostiskt värde för kliniskt arbete inom nötkreatursmedicinen.

(Received August 23, 1973).

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