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GLUTARALDEHYDE AND FORMOL-GEL TESTS IN BOVINE TRAUMATIC PERITONITIS

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LIBERG, PER: *Glutaraldehyde and formol-gel tests in bovine traumatic peritonitis*. Acta vet. scand. 1981, 22, 78—84. — Eighty-two cows with traumatic peritonitis were examined by means of rapid tests for estimation of the blood fibrinogen and immunoglobulin status. The examination was made with this disease as a model in order, to study the possibilities offered by these rapid tests of an improved basis for clinical assessment of the stage and intensity of inflammatory processes in disease. According to their reaction to the tests the cases were divided into 6 reaction groups. All groups exhibited different blood protein patterns, which may be considered to reflect different intensities and/or stages of the disease. A proposed classification is discussed.

bovine plasma proteins; glutaraldehyde test;
formol-gel test; acute and chronic inflammations;
traumatic peritonitis.

Traumatic peritonitis is characterized patho-anatomically by a usually well demarcated purulent inflammation. The clinical diagnosis is not difficult in typical cases.

The course of the disease depends on the degree and site of the penetration. In most cases the clinical symptoms disappear within a few days. If the disease proceeds to a chronic stage, there is a greater variation of clinical manifestations without clear-cut symptoms.

Blood protein determination generally provides valuable information about the inflammatory process. Electrophoretic protein separation has been used to characterize the inflammatory blood protein changes in traumatic peritonitis (*Zimmerli 1955, Björck & Jacobsson 1964, Miclaus et al. 1973, Liberg 1977, a. o.*) The changes observed accord essentially with those arising in the

majority of other inflammations. At an early stage certain α -globulins and fibrinogen increase, while the albumin diminishes. At later stages there is an increase primarily of β_2 - and γ -globulin, while the hypoalbuminaemia persists.

The glutaraldehyde and formol-gel tests are simple, semi-quantitative, rapid methods for detecting hyperimmunoglobulinaemia and hyperimmunoglobulinaemia plus hyperfibrinogenemia in cattle (*Liberg 1973, 1978*). The tests have proved reliable and well suited for use in practice.

The present examination was made in order to illustrate the possibilities offered by these rapid tests to obtain information about the intensity and duration of an inflammation with traumatic peritonitis as a model.

MATERIAL AND METHODS

The material consisted of 82 dairy cows with the clinical diagnosis of traumatic peritonitis. According to anamnestic duration the material was divided into 2 stages of disease: ≤ 7 days = acute stage, $n = 56$, and > 7 days = chronic stage, $n = 26$. Seventy-three per cent of the animals in acute stage had a duration of 0—3 days, 88 per cent of the animals in chronic stage a duration of at least 10 days. Twenty-seven animals (33 %) were emergency-slaughtered and necropsied. The clinical diagnosis was confirmed in all cases necropsied. Thirty clinically healthy cows were used as reference material.

The total protein concentration in serum was determined by the biuret method and albumin spectrophotometrically with bromocresol green (*Doumas et al. 1971*). The total globulin concentration was calculated on the basis of the total protein and albumin values. Fibrinogen in plasma was determined after coagulation with thrombin (*Liberg 1978*). The formol-gel test (FR) in serum and the glutaraldehyde test (GLA) in whole blood were performed as described by *Liberg et al. (1975)*.

On the basis of the rapid test reactions the animals were divided into the following groups: I: both reactions negative, II: GLA positive (0—15 min), FR negative (24 h—), III: GLA negative (15 min—), FR positive (0—24 h), IV: both reactions slowly positive (GLA: 3—15 min, FR: 1—24 h), V: GLA quickly and FR slowly positive (GLA: 0—3 min, FR: 1—24 h), VI: both reactions quickly positive (GLA: 0—3 min, FR: 0—1 h).

Confidence limits were used to evaluate possible differences among group means (Fig. 1).

RESULTS

Table 1 shows the numbers of animals in the different groups and the distribution according to anamnestic duration. Groups I and II with both reactions negative and with only GLA positive, respectively, comprised mostly the anamnestic acute stage, whereas the proportion of chronic stage cases increased the quicker the two tests showed positive reactions.

Table 1. Number of cases of traumatic peritonitis grouped according to types of reaction to GLA and FR tests, with breakdown within each group according to anamnestic duration. GLA positive (0—15 min), GLA slowly positive (3—15 min), GLA quickly positive (0—3 min); FR positive (0—24 h), FR slowly positive (1—24 h), FR quickly positive (0—1 h).

| Anamnestic duration | Group I | | II | | III | | IV | | V | | VI | |
|---------------------|---------|------|------|------|------|-------------|--------------|-------------|--------------|--------------|--------------|--|
| | GLA | neg. | pos. | neg. | pos. | slowly pos. | quickly pos. | slowly pos. | quickly pos. | quickly pos. | quickly pos. | |
| < 7d | | 10 | 11 | 1 | 13 | 9 | 12 | | | | | |
| > 7d | | 1 | 1 | 2 | 6 | 5 | 11 | | | | | |
| Total | | 11 | 12 | 3 | 19 | 14 | 23 | | | | | |

Fig. 1 shows plasma fibrinogen and serum globulin for the different groups except group III, which was excluded since it consisted of only 3 animals. Cows with both reactions negative (group I) showed a moderate increase of fibrinogen in comparison with the reference material. The globulin value was normal. Cows with positive GLA but negative FR (group II) showed a strong increase of fibrinogen, while the globulin concentration was still normal. Cows with both reactions slowly positive (group IV) showed a strong increase of fibrinogen and moderate increase of globulin. Cows with GLA quickly positive and FR slowly positive (group V) had a very heavily increased fibrinogen concentration, whereas the mean globulin concentration was at the same level as group IV. Cows with both reactions quickly positive (group VI) showed very strong increases of both fibrinogen and globulin.

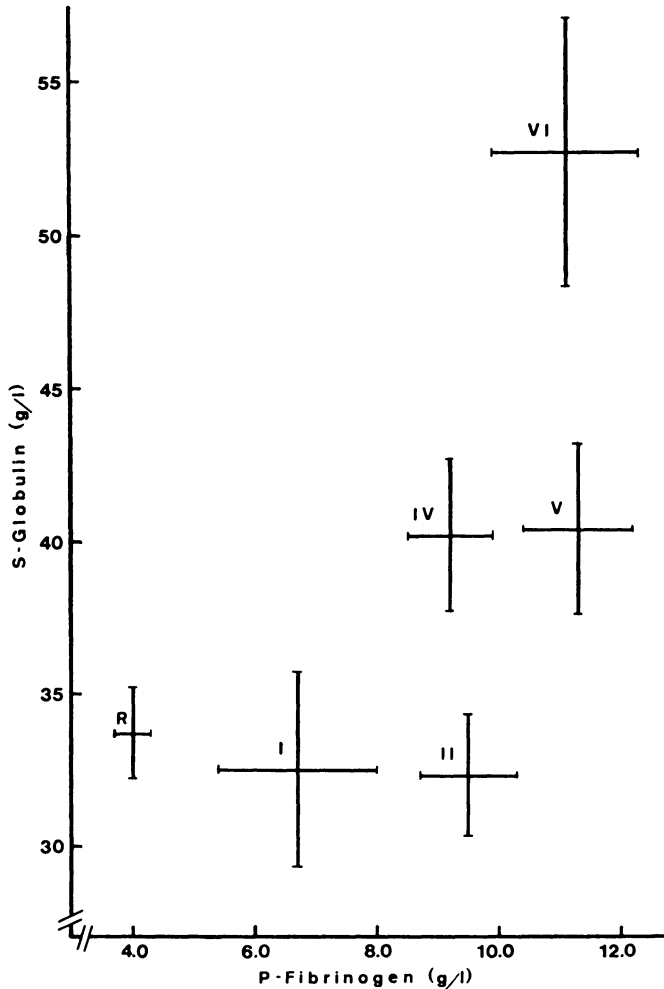


Figure 1. Plasma fibrinogen and serum globulin (95 % confidence limits of the mean) in healthy cows (R) and in sows with traumatic peritonitis grouped according to GLA and FR test reactions: I: Both reactions negative, II: GLA pos. (0—15 min), FR neg. (24 h—), IV: GLA slowly pos. (3—15 min), FR slowly pos. (1—24 h), V: GLA quickly pos. (0—3 min), FR slowly pos., VI: GLA quickly pos., FR quickly pos. (0—1 h). Group III not included because of too few cases.

DISCUSSION

The results are largely similar to those in an earlier study made on a less specified disease material and with a smaller number of groups (*Liberg 1978*).

Cows with both reactions negative and cows with only GLA positive showed normal globulin levels but a significant increase of fibrinogen in comparison with the reference material. The difference in fibrinogen between groups I and II was significant. Fibrinogen is an acute-phase protein. An initial rise of the plasma level is observable already within 24 h after a tissue injury (*Lau-rell et al.* 1976). Groups I and II may therefore be considered to reflect a primary inflammatory blood protein reaction and represent an acute stage of disease. The difference in fibrinogen between groups I and II may be regarded as an expression of the existence of an early and late reaction, respectively, in an acute stage.

Group III was not analysed since it comprised only 3 animals. In general, however, the rapid tests used are so designed that FR becomes positive at a rather lower γ -globulin level than GLA. In animals with group III reaction a smaller increase of γ -globulin but normal fibrinogen level may be expected. This combination of test reactions should therefore reflect inflammatory processes of very low intensity. Such processes are likely uncommon in traumatic peritonitis, which might explain the low frequency.

Groups IV and V with moderate increase of γ -globulin in both groups may be considered to reflect an early secondary inflammatory blood protein reaction and represent a subacute stage of disease. The difference in fibrinogen concentration between these groups would appear to be an expression of a longer duration of disease and perhaps also a rather higher intensity in group V. Groups IV and V might be called the first and second degree, respectively, of a subacute stage of disease.

Group VI, with a heavy increase both of γ -globulin and fibrinogen, reflects the secondary inflammatory blood protein reaction and thus represents the clearly chronic stage of the disease.

In principle there is no difference between traumatic peritonitis and other inflammations of a purulent character. It should therefore be possible, on the basis of the rapid test reactions, to classify such inflammatory processes in cattle into at least the 6 stages of reaction defined in this study.

The anamnestic duration of a disease generally constitutes a basis for clinical assessment of the stage of disease. In most cases there should be fairly close correlation between anamnestic dura-

tion and stage of inflammation measured by the blood protein pattern, which is also true in the present material (Table 1). But there is always a risk of faulty judgement, especially in diseases with periodical course or with high risk of recurrence. An exacerbating stage of a chronic disease, e.g. traumatic peritonitis, may therefore be readily interpreted as an acute stage if the assessment is based solely on anamnestic duration. From Table 1 it will be seen that the cases in group VI were fairly evenly divided between those with shorter and longer anamnestic duration. The greatly elevated globulin values, however, suggest that all cases in this group had a course of disease longer than 7 days. This was confirmed by the existence of clearly chronic inflammatory changes in all necropsied cases (5 of 12) with shorter anamnestic duration than 7 days. It thus appears manifest that several of the cases in group VI had the character of exacerbating chronic traumatic peritonitis.

In conclusion it may be said that the glutaraldehyde and formaldehyde tests in parallel use give a good reflection of the fibrinogen and immunoglobulin status in the blood and thus impart important information to the clinician about the character and course, not only of traumatic peritonitis, but also of several other inflammatory processes, at least those with bacterial background.

REFERENCES

- Björck, G. & S. O. Jacobsson*: Elektrophoretische Untersuchung des Blutserums von Kühen mit chronischer traumatischer Peritonitis. (An electrophoretic study of the blood serum of cows with traumatic peritonitis. III Int. Meet. Diseases of Cattle. Copenhagen 1964, pp. 382—388.
- Doumas, B. T., W. A. Watson & H. G. Biggs*: Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. chim. Acta* 1971, 31, 87—96.
- Laurell, C.-B., B. Lund & B. Nosslin*: Klinisk kemi i praktisk medicin. (Clinical chemistry in practical medicine). Studentlitteratur, Lund, Sweden 1976.
- Liberg, P.*: The formol-gel reaction in cattle. *Acta vet. scand.* 1973, 14, 712—722.
- Liberg, P., B. Pehrson & M. Sandholm*: The value of the glutaraldehyde and formaldehyde tests in evaluation of the globulin level in bovine blood. *Acta vet. scand.* 1975, 16, 336—343.
- Liberg, P.*: Agarose gel electrophoretic fractionation of serum proteins in adult cattle. II. A study of cows with different diseases. *Acta vet. scand.* 1977, 18, 335—348.

- Liberg, P.*: 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—421.
- Miclaus, I., G. Espersen & P. Hjorth*: Plasma protein composition in cattle affected with acute peritonitis. *Nord. Vet.-Med.* 1973, 25, 570—574.
- Zimmerli, J.*: Die Eiweissfraktionen im Blutserum des Rindes in Abhängigkeit von verschiedenen chronischen Krankheiten insbesondere der Tuberkulose. (The relation of serum proteins in cattle to different chronic diseases, particularly tuberculosis). *Z. Tierzücht. Zücht.-Biol.* 1955, 64, 355—380.

SAMMANFATTNING

Glutaraldehyd- och formol-gel-test vid traumatisk peritonit hos nötkreatur.

Glutaraldehydtest (GLA) och formol-gel-test (FR) utfördes för uppskattning av fibrinogen- och immunglobulinstatus i blod på 82 kor med traumatisk peritonit. Fallen indelades efter testutslag i 6 olika reaktionstyper. Testreaktionerna korrelerades till plasmafibrinogen- och serumglobulinkoncentrationer. Mot bakgrund av kända principiellt olika blodproteinreaktionsmönster vid inflammatoriska processer tolkades sjukdomsstadium och/eller -intensitet vid de olika reaktionstyperna enligt följande:

I. Båda tester negativa: akut, tidig reaktion; II. GLA positivt, FR negativ: akut, sen reaktion; III. GLA negativt, FR positiv: låg intensitet; IV. Båda tester långsamt positiva: subakut, 1:a graden; V. GLA snabbt, FR långsamt positiv: subakut, 2:a graden; VI. Båda tester snabbt positiva: kroniskt.

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