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AGAROSE GEL ELECTROPHORETIC FRACTIONATION OF SERUM PROTEINS IN ADULT CATTLE

II. A STUDY OF COWS WITH DIFFERENT DISEASES*

By

Per Liberg

LIBERG, PER: *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. — In 283 dairy cows suffering from internal disorders the serum proteins were studied by agarose gel electrophoresis supplemented by total protein and albumin determinations. The clinical diagnoses could be grouped according to protein pattern. Group 1 (abomasal displacement and traumatic muscle injury) did not appreciably affect the serum protein concentrations and represented primarily non-inflammatory diseases or diseases of non-infectious origin. Group 2 (leukosis) occupied an exceptional position, with heavy lowering of albumin unaccompanied by a corresponding rise of total globulin. The γ-globulin concentration was significantly lowered. Group 3 (acute traumatic peritonitis) represented an acute inflammatory process with increase chiefly of the α-globulins, while the γ-globulin concentration was normal. Group 4 (chronic traumatic peritonitis, summer mastitis, chronic subclinical mastitis, chronic laminitis and polyarthritis, urinary tract infection, abscess, and subacute-chronic pneumonia) comprised diseases chiefly of chronic inflammatory character and with infectious origin. Especially characteristic was the heavy rise of globulins in general and of γ-globulin in particular. In most cases there was a large increase also of the α- and β-globulin fractions.

agarose gel electrophoresis; bovine serum proteins; acute and chronic inflammations.

Reaction patterns for serum proteins in different diseases in man were first presented by *Wuhrmann & Wunderly* (1952). These patterns were arranged according to variations in the globulin fractions. Improvements of the electrophoretic technique in recent years have increased our knowledge of individual pro-

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teins in the serum pattern. Even if specific determinations of individual proteins have meant a great advance in plasma protein diagnosis, there has been no reason entirely to abandon the screen electrophoresis technique. The electrophoretic pattern provides important supplementary information to the determinations of single serum proteins. Agarose gel electrophoresis gives good resolution of the components and the possibility of identifying several of the protein zones without producing too difficultly interpreted patterns, as is the case with so-called high-resolution electrophoresis techniques. Screen electrophoresis is important, for instance, for assessing the immunoglobulin distribution, for which the picture gives special information not obtainable by quantitative determination.

Ever since the investigations of *Wuhrmann & Wunderly*, different reaction patterns for serum proteins have been studied in numerous clinical conditions in man. On the other hand rather few studies have been made of the relation between serum protein pattern and clinical symptoms in domestic animals including cattle. Most publications comprise small animal materials and describe only few cases of illness. The first major investigation of serum proteins in domestic animals by electrophoresis was made by *Boguth* (1954). He established different reaction patterns for serum proteins in the same way as *Wuhrmann & Wunderly* had earlier done for man. Among his findings were that an acute inflammation, especially in pig and dog, was reflected in an increase of α-globulin, a subacute inflammation in an increase of α- and γ-globulin, and a chronic inflammation in an increase of γ-globulin. *Boguth* examined serum from cattle with tuberculosis and observed an increase of the γ-globulins with corresponding reduction of albumin.

Bendixen (1954) studied serum from cows with chronic inflammatory conditions and observed an increase of varying size only in the γ-globulin fraction. *Teichmann* (1958) noted a reduction of the albumin concentration with simultaneous rise of chiefly the α-globulin fraction in inflammatory and purulent processes. *Morcos* (1960) studied the serum protein pattern in cattle with ulceration of the sole. These animals displayed hypoalbuminaemia and hyper-γ-globulinaemia. *Schotman* (1962) grouped different clinical states according to serum protein pattern and concluded that the protein pattern gives valuable information as to whether an animal is suffering from an acute

or chronic inflammatory process. *Björck & Jacobsson* (1964) compared chronic traumatic peritonitis, with and without abscesses, with the serum protein pattern. The total protein concentration, the α- and γ-globulin fractions were increased and the albumin/globulin ratio lowered in cows with abscesses. In cases without abscesses there was an increase of β-globulin. Hypoalbuminaemia was present in both groups.

Åberg (1957) compared the electrophoretic pattern in leukotic and healthy cows. He found no statistically significant differences. *Greve* (1957) likewise studied leukotic animals and observed hypoalbuminaemia with corresponding increase of the α-, β- and γ-globulin fractions. *Matthaeus & Straub* (1965) found that individual protein fractions showed large variations in leukotic animals. They observed no uniform pattern nor any pattern specific to the state or the severity of the disease.

Nilsson (1963) studied laminitis in cattle and, in cases of acute laminitis, found increased α_1-, α_2- and γ-globulin fractions. The albumin concentration and the A/G ratio were lower than normal. In subacute laminitis the α_1 and γ-globulin concentrations were increased. The total protein concentration was normal in both conditions.

The purpose of the present investigation was to study changes in the serum protein pattern in different diseases of cattle, using primarily agarose gel electrophoresis supplemented by total protein and albumin determinations.

MATERIALS AND METHODS

Animals

The material consisted of 283 dairy cows with internal disorders which were subjected to ambulatory care and examination. With few exceptions the animals were of Swedish Red-and-White or Swedish Friesian breed or crosses between the two. The diagnoses were established by clinical examination and routine laboratory analyses. In many cases the diagnoses were confirmed by special analyses, operation or necropsy. The breakdown of the material was as follows:

I *Displacement of abomasum*

Twenty-seven cases, of which 23 left-sided and four right-sided. Fifteen of the animals were operated upon, nine were necropsied at slaughter.

II *Traumatic muscle injury*

Ten cases, all of which with spreadeagling injury after calving. Eight of the animals were examined for ASAT activity in serum. All had abnormally elevated activity. Seven of the diagnoses were confirmed at slaughter.

III *Leukosis*

Twenty-seven cases, all of which were necropsied. Eleven cases were confirmed histopathologically.

IV *Acute-subacute traumatic peritonitis*

Thirty-two cases with a history shorter than seven days. Three were confirmed by operation and seven at slaughter.

V *Chronic traumatic peritonitis*

Thirty-two cases with a history longer than seven days, both uncomplicated local and more widespread peritonites. Twenty-five of the cases were necropsied at slaughter.

VI *Summer mastitis*

Forty-one cases, all occurring during the grazing period. Most cases occurred at the time of the first calving. All had serious local, and many also general, symptoms.

VII *Chronic subclinical mastitis*

Forty-nine cases. Bacteriological tests were made on 38 animals, all of which were infected with streptococci and/or staphylococci. The majority had no general clinical symptoms.

VIII *Chronic laminitis and polyarthritis*

Twenty-one cases, 10 of which were judged to be laminitis and 11 polyarthritis. The clinical symptoms were often rather difficult to interpret. Three of the cases were complicated by ulceration of the sole. Two were necropsied. All animals had an anamnesis longer than seven—10 days.

IX *Urinary tract infection*

Twenty-two cases, most of which with a long history. In 17 cases the diagnosis was pyelonephritis, in the remainder "non-specific urinary tract infection". All animals had haematuria with turbid or pussy urine. Bacteriological tests in seven cases showed infection with Corynebacterium bovis renale in six and haemolysing Escherichia coli in one case. Seven of the animals were examined pathoanatomically. All had chronic pyelonephritis.

X *Abscess*

Ten cases, including so-called cold abdominal abscess, abscess in the vaginal wall, severe suppuration in operation wound.

XI *Subacute-chronic pneumonia*

Twelve cases with a history of at least one week. Half of them were necropsied and revealed purulent or suppurating bronchial pneumonia.

Table 1. Summary of statistical comparisons between means for clinically healthy cows and cows with different diseases.

	Absolute concentration									Relative concentration					
	TP	ALB	GLOB	α_1	α_2	inter-α-β	β_1	β_2	γ	α_1	α_2	inter-α-β	β_1	β_2	γ
Abomasal displacement	n.s.	———	++	+	++	++	n.s.	n.s.	++	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Traum. muscle injury	n.s.	———	++	+++	+++	ns.	n.s.	n.s.	+	++	n.s.	n.s.	——	——	n.s.
Leukosis	———	———	n.s.	+++	+++	+++	n.s.	n.s.	——	+++	+++	+++	n.s.	n.s.	———
Acute traum. peritonitis	+++	———	+++	+++	+++	n.s.	+++	+++	n.s.	+++	+++	n.s.	n.s.	n.s.	———
Chron. traum. peritonitis	+++	———	+++	+++	+++	++	+++	+++	+++	+	n.s.	—	n.s.	———	+
Summer mastitis	+++	———	+++	+++	+++	n.s.	+++	+++	+++	+++	n.s.	———	n.s.	——	n.s.
Chron. subclin. mastitis	+++	———	+++	++	+++	n.s.	+++	+++	+++	n.s.	——	n.s.	——	+	+
Chron. laminitis or polyarthritis	+++	———	+++	+++	+++	n.s.	+++	+++	+++	+	n.s.	n.s.	n.s.	n.s.	n.s.
Urinary tract infection	++	———	+++	+++	+++	+++	+++	+++	+++	+++	n.s.	+	———	n.s.	n.s.
Abscess	+++	———	+++	+++	+++	+++	+++	+++	+++	n.s.	———	n.s.	———	n.s.	++
Subacute-chron. pneumonia	+++	———	+++	+++	+++	+++	+++	+++	+++	n.s.	n.s.	n.s.	——	n.s.	n.s.

TP = total protein, ALB = albumin, GLOB = total globulin.

n.s. = no significant change.

+/— = probably significant increase/decrease ($0.05 > P > 0.01$).

++/—— = significant increase/decrease ($0.01 > P > 0.001$).

+++/——— = highly significant increase/decrease ($P < 0.001$).

This pathological material was compared with a normal material comprising 154 clinically healthy randomly selected dairy cows.

Blood samples were centrifuged and serum was stored at —20°C before analysis.

Protein analysis

The total protein concentration was determined by the biuret method and the albumin spectrophotometrically with bromcresol green (*Doumas et al.* 1971). The total globulin content was calculated from the total protein and albumin values.

The technique of electrophoresis followed the method of *Carlström & Liberg* (1975) using agarose gel and a double buffer system.

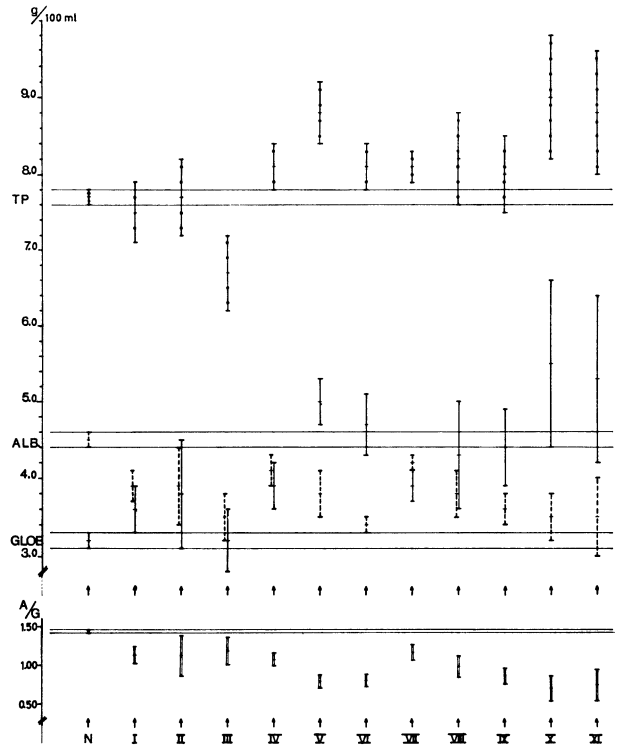

Figure 1. Total protein (TP), albumin (ALB), total globulin (GLOB) concentrations ($\bar{x} \pm 95\ \%$ C.I.) and A/G ratio in clinically healthy animals (N) and animals with different diseases (I—XI).

Gel scanning

The electrophoresis pictures were evaluated in a Beckman spectrophotometer Acta C III with gel scanner and integrator recorder.

RESULTS

Figure 1 shows the total protein, albumin and total globulin concentrations in healthy and diseased cows. Figs. 2 and 3 show the relative and absolute concentrations of the globulin fractions. Fig. 4 illustrates some examples of electrophoretic protein pictures of diseased cows. Table 1 summarizes the statistical comparisons of the individual protein fractions.

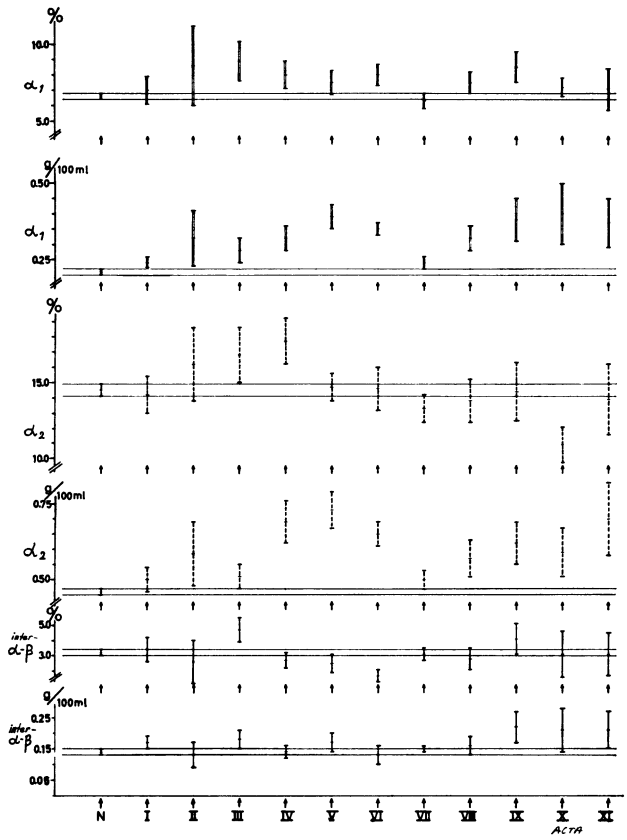

Figure 2. Relative and absolute concentrations ($\bar{x} \pm 95$ % C.I.) of α_1-, α_2- and inter-α-β-globulin in clinically healthy animals (N) and animals with different diseases (I—XI).

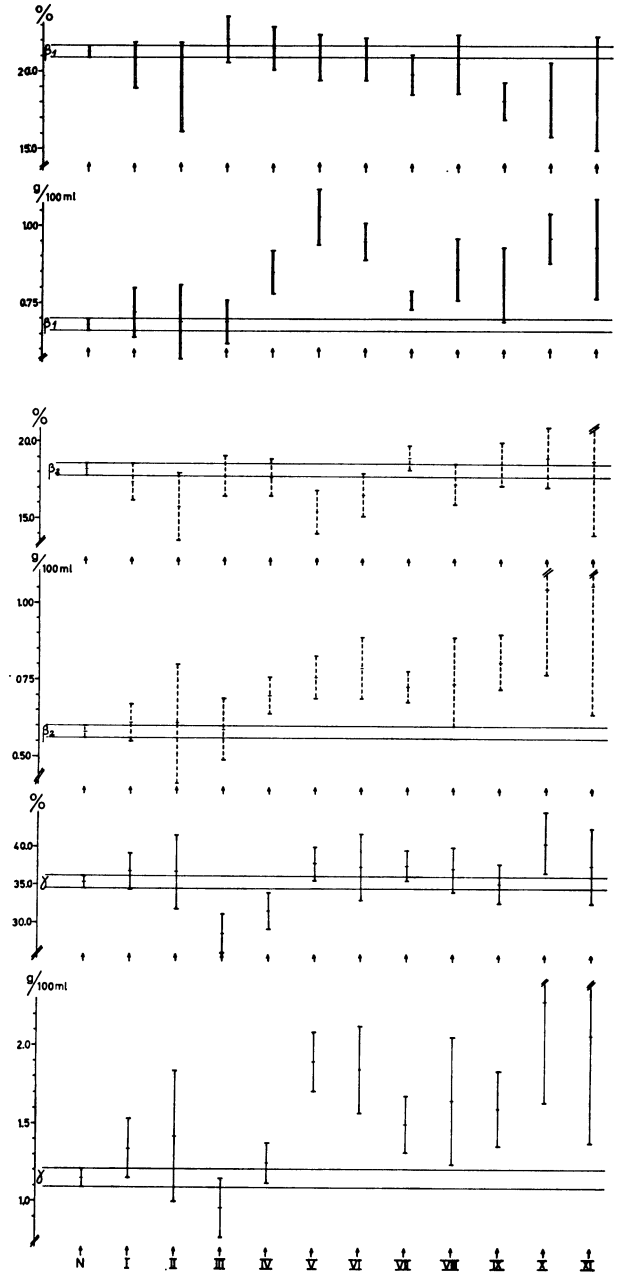

Figure 3. Relative and absolute concentrations ($\bar{x} \pm 95\ \%$ C.I.) of β_1-, β_2- and γ-globulin in clinically healthy animals (N) and animals with different diseases (I—XI).

The albumin concentrations and the A/G ratios were significantly lowered in all diseases studied. Based on the protein pattern in other respects the diagnoses could be divided into groups with special characteristics as follows:

Group 1. Displacement of abomasum and muscle injury.
Moderate rise of total globulin, normal total protein concentration.
Normal relative concentrations for most globulin fractions.
Increase of the absolute α- and γ-globulin concentrations, the latter only slightly to moderately.

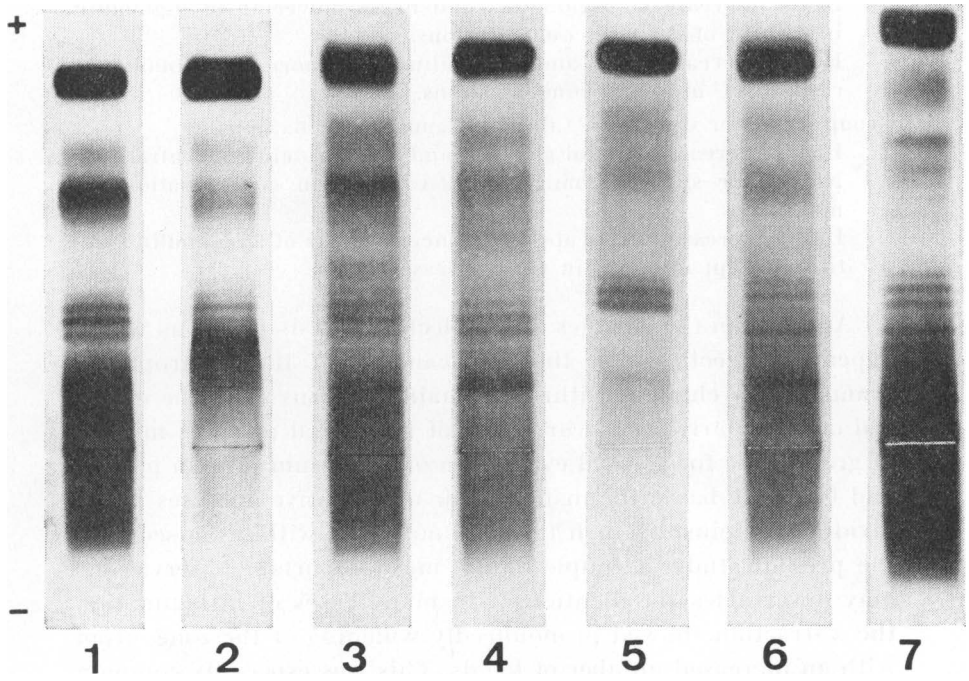

Figure 4. Examples of electrophoretic protein pictures of diseased cows. 1. Very strong hyper-γ-globulinaemia and bilirubinaemia (within the α_2-globulin zone) in chronic traumatic peritonitis. 2. Normal globulin concentration with a pathologic broom-like protein picture within the β-region in acute traumatic peritonitis. 3. Strong hyper-γ-globulinaemia with a heavy increase of α_1-and α_2-proteins and strong bilirubinaemia with widening of the α_2-globulin zone in chronic traumatic peritonitis with jaundice. 4. Strong hyperglobulinaemia and widening of the α_2-globulin zone with several bands, in chronic traumatic generalized peritonitis. 5. Hypoproteinaemia with pronounced hypo-γ-globulinaemia in leukosis. 6. Strong hyperglobulinaemia with extra bands within the cathodal part of the γ-globulin zone (oligoclonal immunoglobulins) in multiple abscesses. 7. Very strong hyper-γ-globulinaemia and sub-division within the α_2-globulin zone in chronic polyarthritis.

Group 2. Leukosis.
Heavily lowered total protein concentration caused by heavy lowering of albumin.
Heavy increase of the relative and absolute α-globulin concentrations.
Moderate to heavy lowering of the relative and absolute γ-globulin concentration.

Group 3. Acute traumatic peritonitis.
Heavy increase of the total globulin and total protein concentrations.
Heavy increase of α-globulins and heavy lowering of γ-globulin in respect of relative concentrations.
Heavy increase of α- and β-globulins with normal γ-globulin in respect of absolute concentrations.

Group 4. Other diagnoses (all of inflammatory character).
Heavy increases of total globulin and total protein concentrations.
Normal or slightly changed relative globulin concentrations in most cases.
Heavy increases of the absolute concentrations of all globulin fractions except inter-α-β in some diseases.

Apart from the changes of the electrophoresis fractions which appeared objectively in the gel scanning of the electropherograms, many changes within the main fractions could be observed only ocularly. Such variations of individual proteins may be of great value for general evaluation of the serum protein picture and form the basis, for instance, for quantitative analyses of individual proteins. Though they did not come within the scope of the present study, a couple of the most important observations may nevertheless be mentioned. In many cases of inflammation the α_2-fraction showed pronouncedly widening of the zone, often with an increased number of bands. This was especially common in serum from animals with traumatic peritonitis, summer mastitis and urinary tract infection. Among the cases of summer mastitis up to five separate protein bands sometimes arose within the α_2-fraction. Rises of oligoclonal immunoglobulins, with the occurrence of a varying number of small distinct bands in the IgG region, were relatively common in cases of summer mastitis among others.

DISCUSSION

When assessing changes of the serum protein composition in a single case of disease it is important to pay attention to the physiological variations. One of the most important causes of

physiological variation is age (e.g. *Liberg* 1977). In the age interval to which most of the present animals belonged, however, the age variation is so slight as to be disregardable.

The diagnoses could be grouped according to serum protein pattern. Group 1 comprised displacement of the abomasum and traumatic muscle injuries, i.e. diagnoses of primarily non-inflammatory character. The total protein concentration was unchanged and a lowered albumin concentration was compensated by a moderate rise of total globulin, caused chiefly by a rise of γ-globulin. The changes in all globulin fractions, however, were numerically relatively slight. The results accord with the observations of other authors that in most cases non-inflammatory diseases or diseases of non-infectious origin do not appreciably affect the serum protein concentrations.

Leukosis (group 2) occupied an exceptional position, with heavily lowered total protein concentration caused chiefly by a heavy lowering of albumin without corresponding increase of the total globulin. The heavy lowering of albumin would appear to be due to increased catabolism, malabsorption and abnormal losses from different organs. The increase of α-globulins indicates an active process. The lowered γ-globulin concentration accords well with the condition in lymphoproliferative diseases in man, in which a diminished immunoglobulin synthesis has often been observed. *Trainin et al.* (1968) and *Trainin & Klopfer* (1971) found that bovine leukosis is accompanied by deficiency of IgM. Serum IgM could not be detected, or was present only in trace quantities, in more than 80 % of leukaemic cattle. The remaining animals had normal or elevated levels of serum IgM. The authors maintained that the absence of IgM was caused by reduced IgM production in spleen and lymph nodes.

Acute traumatic peritonitis (group 3) represented an acute inflammatory reaction. Above all there was a marked rise of the α_1- and α_2-globulin fractions in which the so-called acute phase reactors chiefly occur. The γ-globulin concentration was normal. The increase of β-globulins was slight to moderate. The slight increase in the β_2-globulin fraction may be due to increase of complement in conjunction with the acute inflammatory reaction. *Laurell* (1972) showed that the intensity of the β_2-band in agarose gel electrophoresis reflects the B_{1c}-globulin (C3 factor) concentration.

Group 4 comprised diseases chiefly of chronic inflammatory character and of infectious origin. An especial characteristic of these states was the very heavy increase of globulin in general and of γ-globulin in particular. The summer mastitis cases and some of the urinary tract infections were evaluated clinically as acute conditions. They nevertheless showed very high γ-globulin concentrations. Even if the anamneses were short, the reactive hyper-γ-globinaemia may have been occasioned by the fact that the processes were of longer duration but had had initial phases without clinically observable manifestations.

In most cases a large increase of the α- and β-fractions was also observed. The α-fractions are largely dominated by acute phase reactors. After increase of these fractions the return to the original values may take several weeks. An increase of the α-fractions in this group of diseases, therefore, need not indicate that they have not been of lengthy duration. The increase of the β-globulin fractions was probably due mostly to the fact that, especially in severe hyper-γ-globulinaemia, there is usually an increased immunoglobulin concentration within the entire electrophoretic mobility zone which contains these globulins. For this reason the proportion of the immunoglobulins in the numerical values of the β_1- and β_2-fractions rises, so that the latter poorly reflect the concentrations of transferrin and β_{1c}-globulin. In this context it may be mentioned that ocular inspection may have advantages over objective optical recording. By the former method, for example, attention can be paid to the addition of "background gamma" to the β-fractions. Quantitation of transferrin by electro-immunoassay in sera from cows showed no increase either in acute or chronic inflammatory conditions (*Liberg* to be published). This would seem to confirm that the recorded increases of β_1-globulin were false.

In oligoclonal hyper-γ-globulinaemia which was observed in cases of summer mastitis among others a few antibody-producing cell clones proliferate. They produce electrophoretically homogenous proteins in such quantities that small bands arise within the immunoglobulin zone. This reflects a narrow and usually early antibody response to the antigen stimulation. In more lengthy processes the bands fall together into a polyclonal pattern. The oligoclonal hypo-γ-globulinaemia is common in early and often violent stages of infection. It can therefore serve as guidance in assessing the course of illness and the prognosis. The

oligoclonal pattern in, for example, summer mastitis might be explained by the violence of these inflammations. Persistent oligoclonal pattern in chronic morbid processes has in human medicine been related to malignity.

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SAMMANFATTNING

Agarosgelelektroforetisk fraktionering av serumproteiner hos vuxna nötkreatur. II. Undersökning av kor med olika sjukdomstillstånd.

Hos 283 internmedicinskt sjuka mjölkkor undersöktes serumproteinerna genom agarosgelelektrofores kompletterad med totalprotein- och albuminbestämningar. De kliniska diagnoserna kunde grupperas efter proteinbild: Grupp 1 (löpmagsdislokation samt traumatisk muskelskada) påverkade ej nämnvärt serumproteinkoncentrationerna och representerade primärt icke-inflammatoriska eller icke-infektiösa sjukdomstillstånd. Grupp 2 (leukos) intog en särställning med kraftig albuminsänkning utan motsvarande ökning av totalglobulinet. γ-globulinkoncentrationen var signifikant sänkt. Grupp 3 (akut traumatisk peritonit) representerade en akut inflammatorisk process med ökning av i första hand α-globulinerna medan γ-globulinkoncentrationen var normal. Grupp 4 (kronisk traumatisk peritonit, sommarmastit, kroniska subkliniska mastiter, kronisk laminit och polyartrit, urinvägsinfektion, abscess samt subakut-kronisk pneumoni) innefattade sjukdomar i första hand av kronisk inflammatorisk karaktär och med infektiös genes. Speciellt utmärkande var den kraftiga globulinökningen i allmänhet och γ-globulinökningen i synnerhet. I de flesta fall iakttogs stor ökning också av α- och β-globulinfraktionerna.

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Reprints may be requested from: Per Liberg, the Research Station of the Veterinary Institute, S-532 00 Skara, Sweden.