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ELECTROPHORETIC STUDIES OF NORMAL BOVINE AND GUINEA-PIG SERUM

By

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Quantitative electrophoretic studies of normal bovine serum have been performed by *Bradish et al.* (4), *P. Wehmeyer,* among others. *Wehmeyer* studied the influence of thirst, water intake, and starvation upon the composition of blood from cattle (22), individual differences in the composition of blood from cattle (23, 24) and variations in the plasma protein concentration of the blood etc. as a function of age (25). He also carried out quantitative studies of normal guinea-pig serum (26).

The present paper reports electrophoretic studies of normal cattle and guinea-pig serum by immunoelectrophoresis, agar-gel micro-electrophoresis, and paper electrophoresis.

The protein fractions found by immunoelectrophoresis were characterized by some of their physical-chemical properties, such as solubility, enzymic activity, carbohydrate and lipid content.

MATERIALS AND METHODS

Animal Material

Pooled normal bovine serum from 1—2 years old cattle of red Danish dairy breed, was prepared in the usual way. For purposes of subsequent possibilities of comparison with serum from infected or vaccinated cattle, stalled isolated, the not milk yielding cows was fed exclusively on hay.

Normal guinea-pig serum was prepared by pooling sera from young, adult, non-pregnant guinea-pigs weighing about 400 g.

Five anti-bovine sera were prepared as follows: 5 albino rabbits were injected subcutaneously at two-week intervals with 0.5, 0.5, 1, and 0.5 ml. bovine serum, adding to the first dose 0.5 ml. Freund's adjuvant at the ratio 1:1. Exactly 7 days after the last injection blood was collected. A sixth anti-bovine serum, prepared in an analogous way, was a goat anti-bovine serum.

Moreover, 6 anti-guinea-pig sera were prepared, *viz.* 5 rabbit anti-guinea-pig sera and 1 goat anti-guinea-pig serum.

Salting Out

Salting out with ammonium sulphate was used (*Clausen and Heremans* (5)). The ammonium sulphate concentrations were 1.2, 1.6, 2.0, 2.4, and 2.8 M (pH = 6.8).

5 ml. serum was diluted to 10 ml. with distilled water in order to counteract adsorption. To obtain the above-mentioned salt concentration suitable portions of 4 M ammonium sulphate (pH = 6.8) were added to the serum dilution.

The mixtures were incubated for 24 hours in water bath at 37°C and then centrifuged at 4°C for 20 minutes at 18000 rpm.

The precipitates were washed twice with ammonium sulphate solutions of the same concentration as that used in the precipitation and again isolated by centrifugation. Then, they were dissolved in tap water and diluted, until the volume had been brought up to the initial volume.

The supernatants were united with their appurtenant washes and then concentrated by vacuum dialysis in an apparatus designed by Membranfilter Gesellschaft, Göttingen. During the concentration the fractions were dialyzed against tap water.

Immunoelectrophoresis

Scheidegger's micromethod (17) was used.

Agar-gel micro-electrophoresis was carried out as described by *Wieme* (28).

Paper electrophoresis was done on Whatman No. 1 paper and as described by *Laurell and Skoog* (11).

The electrophoresis slides were stained in all cases for protein with Amido Black 10 B.

Autoradiography was performed in principle as described by *Clausen and Munkner* (7): To 100 µl. serum add 50 µl. of a ferric citrate solution (pH = 5.5) containing the isotope Fe^{59} in a

quantity which gave an activity of 1.5 μC. The ferric citrate solution contained less than 1.5 μg. iron. The radioactive sera were subjected to immunoelectrophoresis. Then, the dried slides were placed, agar side down, on X-ray films for 7—8 days. After exposure of the film and staining of the slides for protein the iron-binding fraction in the serum could be identified.

Special Stains

In addition to ordinary protein staining of electrophoresis slides, special staining was performed in order to identify lipids, glycoproteins, esterases, and oxidases.

Electrophoretic slides were prepared in the way that on the same slide two parallel patterns of serum were run, whereupon one half of the slide was protein stained for reference, while the other half was special stained, so that the electrophoretic situation of the special-stained fractions in relation to the other serum proteins could be accurately established. In paper electrophoresis the procedure was the same.

Lipid Staining was performed by Oil Red O as described by *Wieme* (28), and according to a method described by *Macdonald and Kissane* (12), by which Sudan Black being dissolved in ethyl acetate and propylene glycol and mixed with serum prior to electrophoresis. This method proved applicable in agar-gel micro-electrophoresis, but not in immunoelectrophoresis.

Staining for Esterase Activity. The method was worked out by *Uriel* (20). The enzymes which have possibly been separated by electrophoresis act upon β-naphthyl-acetate which is thereby split. The released β-naphthol reacts with diazo blue, forming a reddish purple pigment.

Inhibition of Esterases. In order further to characterize the esterases, inhibition experiments were performed, using 10^{-3}, 10^{-4}, and 10^{-5} M Physostigmine and 10^{-5} M DFP (diisopropyl-fluorophosphate) (*Pearse* 15)). This makes it possible to classify the esterases into A, B, and choline esterases.

Staining for Oxidase Activity was performed by the method of *Uriel* (20), but modified in the way that dimethyl paraphenyl-enediamine was used as medium.

After being stained, the agar-gel micro-electrophoretic slides were dried in the dark at room temperature, being placed, agar side down, on filter paper. This changed the colour from rose to black, most of it diffusing into the paper without any marked

spread. Thereafter, the plates could be stored sticking to the filter paper.

Staining for Glycoprotein was following paper electrophoresis according to the modified PAS method described by *Aronsson* (2).

RESULTS

Normal Bovine Serum

An agar-gel micro-electrophoretic slide (Fig. 1 D & G) showed 6 protein fractions: Albumin α-1, α-2, β-1, β-2, and γ, β-1 and β-2, however, usually coinciding.

Immunoelectrophoresis of bovine serum can demonstrate 17 fractions (Fig. 2), but by several of the antisera a smaller number of precipitation lines was obtained, not all immunized animals forming antibodies to all bovine serum proteins. The following precipitates were found:

In the γ area γ-1 will be seen as a long precipitation line encroaching upon the α-2 area. In addition there is, in the vicinity of the cathode, a fainter precipitation linie γ-2.

The β-2 area contains β-2-I, β-2-II, and β-2-III. All these precipitates, as well as γ-1, manifest themselves as longish bands indicating that each one contains a number of proteins of different mobility but possessing the same immunologic properties. β-2-II often presents itself as a long S-shaped band whose anodic and cathodic parts vary in intensity.

In the β-1 area there are 2 precipitates: β-1-I and β-1-II.

In the α-2 area there is an α-2-I globulin and a diffuse precipitin line whose situation varies from being hardly diffused towards the antibody reservoir to being like a chord to the line α-2-I, often as in Fig. 2 in the form of a prolongation of the γ-1 line. This fraction is called α-2-II.

In the case of α-1 globulins there are 7 fainter precipitates: α-1-I to α-1-VII. Their identification may be difficult, since their situation and length may vary and they are relatively close together. α-1-IV encroaches upon the α-2 area where it meets β-1-I in the middle of α-2-I. This facilitates the identification in the anodic area.

Closest to the anode there is a large, ellipsoid precipitin line representing albumin. No precipitates were found in the prealbumin area.

By means of fractionated salting out with ammonium sulphate and subsequent immunoelectrophoresis of the precipitates

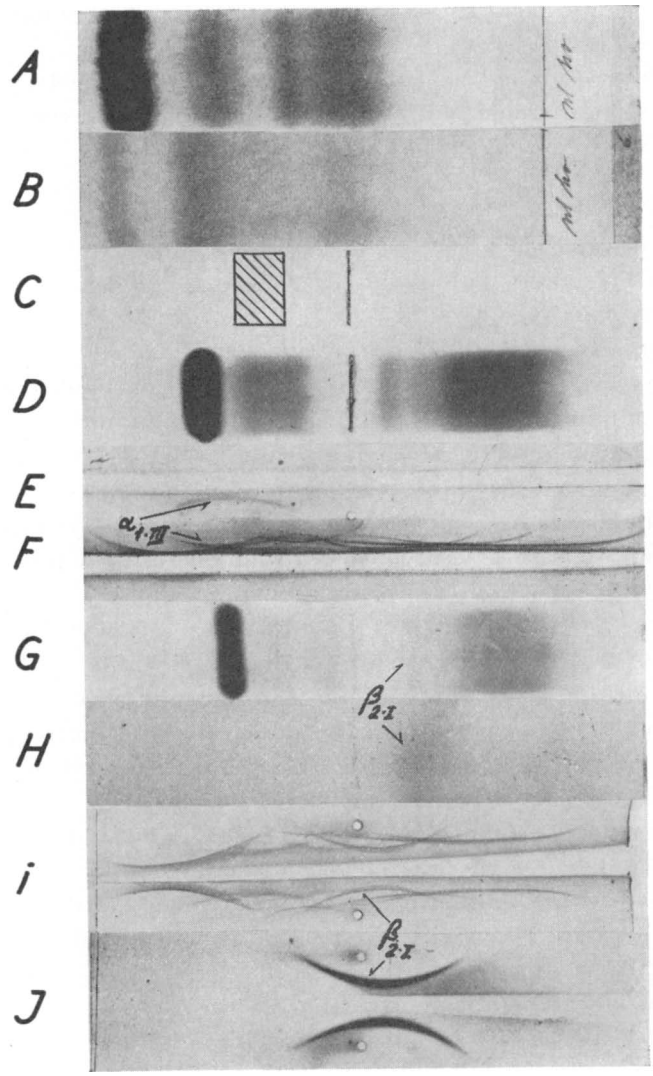

Fig. 1. (cattle serum) — A. Paper electrophoresis stained for proteins. — B. Identical with A stained for glycoproteins. — C. & D. Agar-gel micro-electrophoresis slide, C stained for lipids, D stained for proteins. — E. & F. Immunoelectrophoresis slide, E stained for esterases, F stained for proteins. — G. Agar-gel micro-electrophoresis of serum mixed with Fe^{59}-solution stained for proteins. — H. Autoradiography of G. — I. Immunoelectrophoresis of the same serum as used by G. — J. Autoradiography of I.

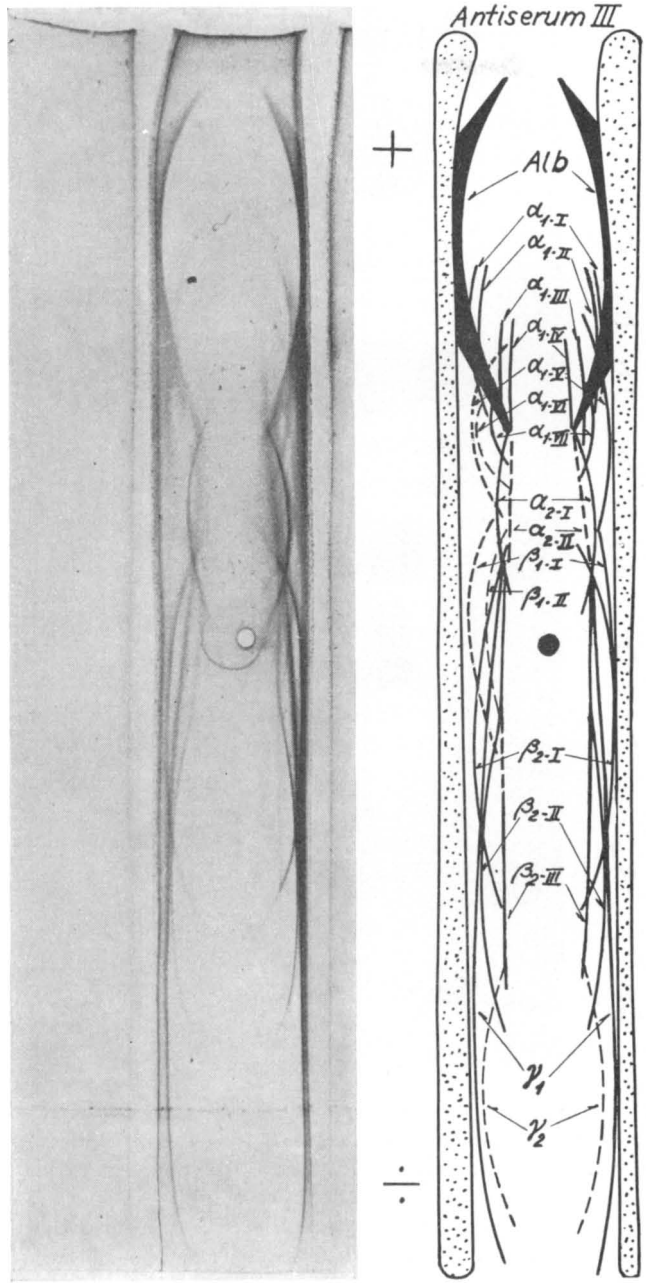

Fig. 2. Immunoelectrophoresis of cattle serum.

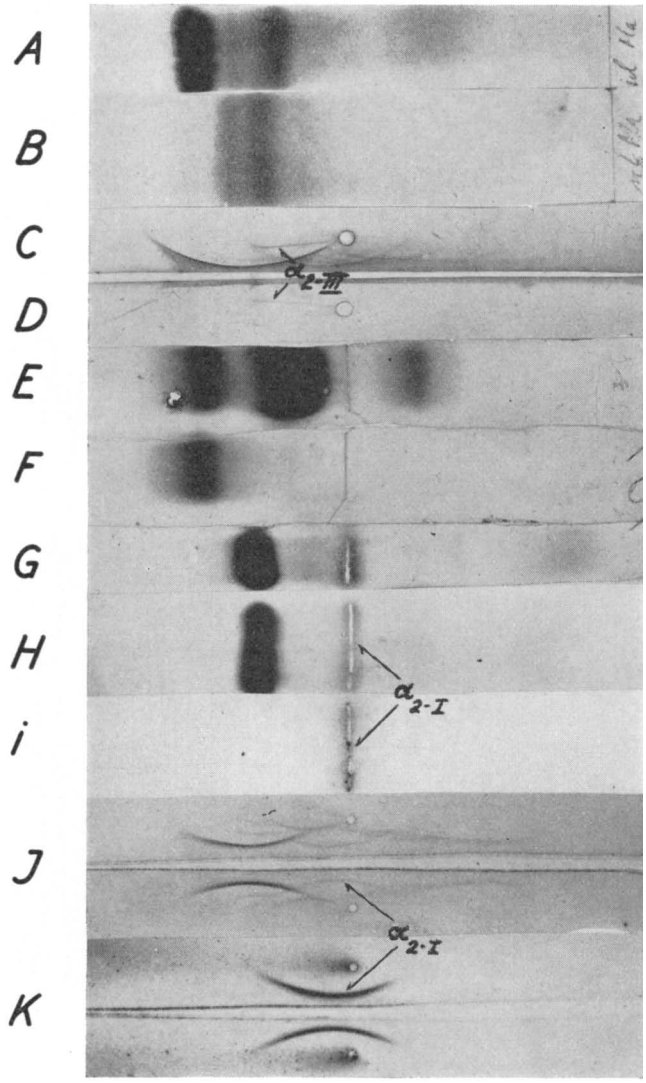

Fig. 3. (guinea pig serum) — A. Paper electrophoresis stained for proteins. — B. Identical with A stained for glycoproteins. — C. & D. Immunoelectrophoresis slide, C stained for proteins, D stained for lipids. — E, F & G. Agar-gel micro-electrophoresis with 3 patterns, E stained for esterases, F stained for esterases following inhibition with 10^{-5} DFP, G stained for proteins. — H. Agar-gel micro-electrophoresis of serum mixed with Fe^{59}-solution. — I. Autoradiography of H. — J. Immunoelectrophoresis of the same serum as used by H. — K. Autoradiography of J.

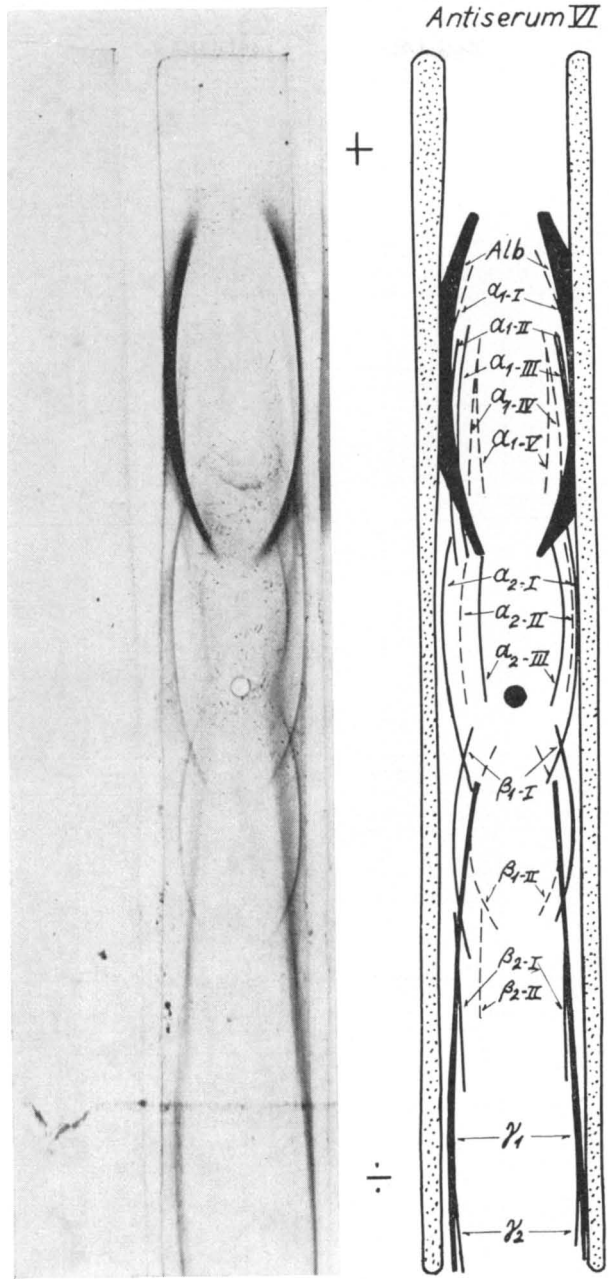

Fig. 4. Immunoelectrophoresis of guinea pig serum.

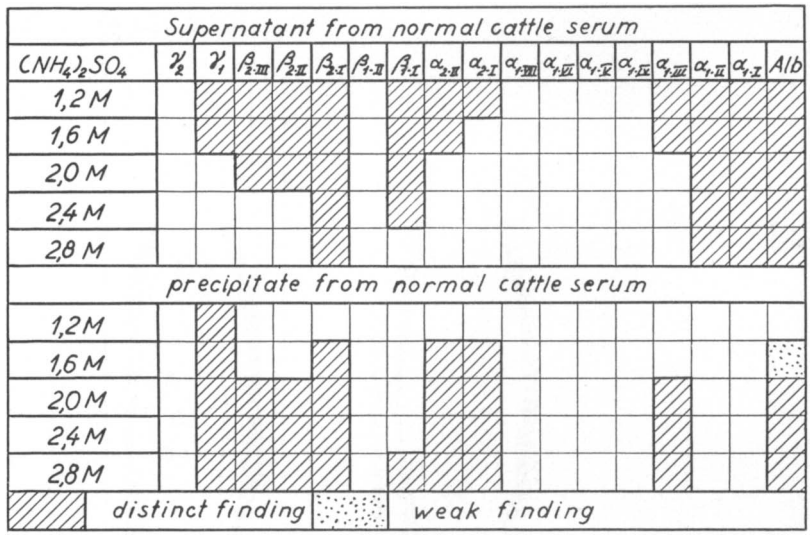

Table 1.

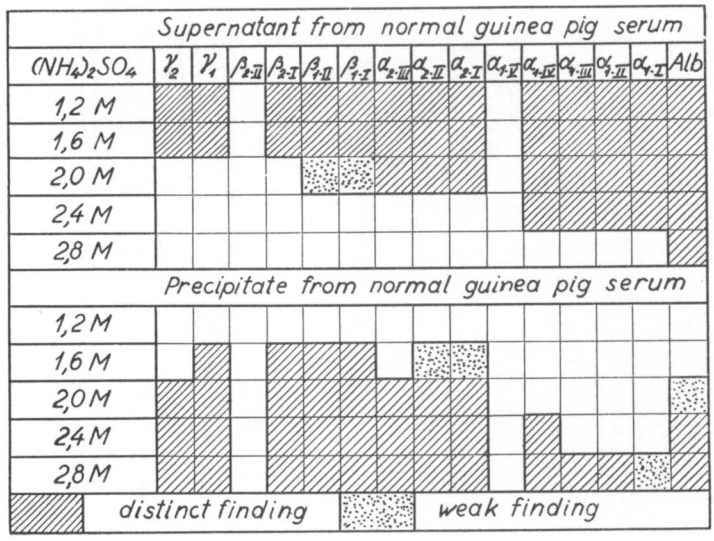

Table 2.

and supernatants, an attempt was made to determine the salting-out limits of the fractions observed in immunoelectrophoresis (Table 1). The following protein groups were found:

(1) A group of globulins comprising γ-1, γ-2, β-2-II, and β-2-III. γ-2, manifesting itself as a very faint line on immunoelectrophoresis of whole serum (cf. Fig. 2), could not be definitely demonstrated upon immunoelectrophoresis of the fractions obtained by salting out. Thus, γ-2 was found only in small quantities in normal serum, or else the fraction has only weak antigenic properties. γ-1 was precipitated partially at 1.2 M and completely at 1.6 M ammonium sulphate. The precipitate from 1.2 M was found, upon immunoelectrophoresis, to consist exclusively of the partially precipitated γ-1 fraction. β-2-II and β-2-III were completely precipitated at 2.0 M.

(2) β-2-I globulin, extending from the middle of the γ area to the α-2 area, was found in all precipitates and supernatants except the precipitate from 1.2 M.

(3) Fainter precipitation lines indicated β-1-I and β-1-II lying as almost concentric lines, the former being closer to the antibody reservoir. On immunoelectrophoresis it was difficult to distinguish between these fractions owing to their concentric situation, the distance of the lines from the antibody reservoir varying somewhat depending on the agar-gel charge and diffusion conditions.

(4) A group consisting of α-2-I and α-2-II. α-2-I was completely precipitated at 1.6 M. α-2-II started to precipitate at 1.6 M and had been completely precipitated at 2.0 M.

(5) The α-1 area difficult to survey because of the numerous superpositions. The fractions appear to follow α-1-III during the salting-out procedure.

α-1-VI and α-1-VII are seen as short, rather faint lines with a long radius of curvature.

α-1-V is a short line cathodic part crosses, and in some cases merely touches α-2-I, while its anodic part generally coincides with the cathodic part of albumin. It was impossible to determine the salting-our properties of α-1-V in detail.

α-1-IV is a very faint line, in most cases impossible to detect in the salting-out fractions.

α-1-III is a relatively long line whose cathodic part issues from the middle of α-2-I and whose anodic part crosses α-1-VI and α-1-VII. α-1-III is completely precipitated at 2.0 M.

α-1-II and α-1-I manifest themselves as two almost equally distinct and equally long lines. These lines are quite close together, manifesting themselves, occasionally as a single, somewhat diffuse line. They are not precipitated, not even at a salt concentration of 2.8 M.

A difficulty in identifying the cathodic parts of all α-1 lines is that they coincide with or are situated quite close to the cathodic part of albumin. The latter occurs in precipitates and supernatants at all concentrations of ammonium sulphate, but only small quantities in precipitates from 1.6 M ammonium sulphate.

Oxidase Activity

Agar-gel micro-electrophoresis of normal bovine serum compared with normal human serum disclosed slight oxidase activity in the α-2 area. The reaction could not be verified in immunoelectrophoresis, presumably because in this procedure the quantity of serum is considerably smaller.

Staining for Glycoprotein

After paper electrophoresis there was a positive reaction in 3 areas (Fig. 1 A & B), staining in the α-2 area being strongest. The α-1 and albumin areas showed fainter PAS reaction (indicating the presence of a diol group). The staining of albumin is presumably a false reaction (2).

Staining for Lipoprotein

Agar-gel micro-electrophoresis disclosed a lipoprotein in the borderline area between the α-1 and α-2 fractions (Fig. 1 C). The fraction was visualized on staining with Oil Red and with Sudan Black. The lipid-stained area was ill-defined in the anodic as well as cathodic parts. The staining was distinct, but diffucult to photograph. Therefore, the area is hatched in Fig. 1 C.

Immunoelectrophoresis showed that in the serum there are 2, possibly 3 lipoproteins. However, the precipitates containing lipid were so faint as to be non-identifiable on a photograph. One of these lipid fractions is α-1-III. Presumably, however, both α-1-III and α-1-IV are lipoprotein precipitates.

In addition, α-2-II manifests itself as a lipoprotein. The ill-defined lipoprotein band found in the α-1 and α2 area on lipid

staining following agar-gel micro-electrophoresis is accordingly explicable as a consequence of overlapping.

Esterase Activity

Agar-gel micro-electrophoresis showed a strong activity in the α-1 area. Correspondingly, immunoelectrophoresis (Fig. 1 E) shows where the precipitation line representing α-1-III exhibited a strong activity. However, there may possibly be a question of α-1-IV, since, as already mentioned, these lines are very close together.

In addition, there is after agar-gel micro-electrophoresis a faint activity in the γ area not demonstrable by immunoelectrophoresis.

On inhibition by 10^{-5} M DFP, the activity in the α-1 area remains undiminished, while the activity in the γ area disappears.

Inhibition with physostigmine in the concentrations 10^{-5}, 10^{-4}, and 10^{-3} M entails a gradual attenuation of activity in the α-1 area without its ever disappearing. There is just a trace of activity in the γ area after inhibition with 10^{-5} M physostigmine, while 10^{-4} and 10^{-3} M physostigmine inhibit this fraction completely.

Autoradiography

β-2-I showed iron-binding properties analogous to human β-1-transferrin (Fig. 1 G, H, I, J).

Normal Guinea-pig Serum

After agar-gel micro-electrophoresis of normal guinea-pig serum there are 6 or 7 fractions (Fig. 3 G & H): Albumin, α-1, α-2, β-1, β-2, β-3, and a γ fraction, the latter in the form of a relatively broad band. α-1 and the three β fractions are so faint that they are difficult to discern on a photograph.

Immunoelectrophoresis revealed 15 fractions (Fig. 4):

(1) Two confluent precipitates in the anodic part: γ-1 and γ-2. The latter is considerably fainter than γ-1 whose anodic part reached close to the application hole.

(2) Two β-2 globulins, the anodic part of β-2-I coinciding with or crossing γ-1 in an acute angle. β-2-II developed as a very faint, almost straight line, often impossible to discern. β-2-II was at a relatively great distance from the antibody reservoir.

(3) Two β-1 globulins, β-1-I and β-1-II, of an almost concentric situation. As a rule, β-1-II touches the anodic part of γ-1.
(4) Three α-2 globulins, α-2-I, α-2-II, and α-2-III, in the form of 3 concentric lines, α-2-II being the faintest. In its cathodic part α-2-I intersects both β-1-I and β-1-II. α-2-II is less marked than α-1-I. The shape and situation of α-2-III may vary in relation to the antibody reservoir. Frequently, it presents itself as an almost straight line issuing from the application hole (Fig. 3 C & D).
(5) Five, usually faint α-1 globulins, α-1-I, α-1-II, α-1-III, α-1-IV, and α-1-V. These lines are close together, and their situation in relation to each other may vary somewhat. The anodic part of lines is situated in the anodic part of albumin, whereas in the cathodic parts they cross the cathodic part of albumin. α-1-II and α-1-III are close together and often coincide. α-1-IV is a relatively flat line. The situation of α-1-V is extremely varied. Owing to its esterase activity (*vide infra*) its varying situation may be traced. On agar-gel micro-electrophoresis it always presents itself as prealbumin, while in immuno-electrophoresis it may be present as either prealbumin or α-1 globulin, usually as the latter. α-1-I and α-1-V do not always manifest themselves and thus must be assumed to be weak antigens.

On salting-out with ammonium sulphate the following precipitation limits were found:

(1) A group of globulins having a mobility corresponding to the γ area, comprising γ-1, γ-2, β-2-I, and β-2-II, the last-mentioned being recovered on salting-out in only one case, *viz.* in the supernatant from 1.2 M ammonium sulphate. The others start precipitating at 1.6 M, being completely precipitated at 2.0 M.
(2) Two β-1 proteins, β-1-I and β-1-II, both beginning to precipitate at 1.6 M and being present as traces in the supernatant from 2.0 M, while they have been completely precipitated at 2.4 M.
(3) Still three α-2 proteins, α-2-I, α-2-II, and α-2-III which also occur at the same time during precipitation, starting at 1.6 M, though only to a slight extent, and being completely precipitated at 2.4 M.

(4) Five α-1 proteins, α-1-I, α-1-II, α-1-III, α-1-IV, and α-1-V, which are precipitated by 2.8 M ammonium sulphate.
(5) Albumin was found in all supernatants. In the precipitate from 2.0 M albumin was present as traces, while in the precipitates from 2.4 and 2.8 M it was found in a concentration so high that the precipitation lines were of the same intensity as in the supernatants.

Oxidase Activity

After agar-gel micro-electrophoresis of guinea-pig serum, compared with normal human serum, there was a faint oxidase activity in the α-2 area. This activity could not be demonstrated by immunoelectrophoresis in which a smaller quantity of serum is used.

Glycoprotein Content

After paper electrophoresis the α-1 and α-2 areas stained with PAS (Fig. 3 B), indicating the presence of glycoproteins in these areas.

Lipoprotein Content

After agar-gel micro-electrophoresis a positive lipid staining was obtained in the entire anodic area, from the application hole to the albumin. Furthermore, the albumin and prealbumin areas showed more marked lipid staining.

Immunoelectrophoresis showed marked lipid staining of α-2-III (Fig. 3 D) and a not always reproducible staining of α-1-V. In addition, there was staining of the chylomicron fraction which on immunoelectrophoresis manifested itself as a short, diffuse, rectilinear zone, issuing anodically from the application hole. The chylomicron fraction is not of antigenic character.

Esterase Activity

After agar-gel micro-electrophoresis a pronounced reaction is seen in the prealbumin area, in the α-1-α-2 area, and in the β-2 area (Fig. 3 E).

Upon inhibition with 10^{-5} M DFP the activity in the α-1-α-2 area and in the β-2 area disappears, while in the prealbumin area it remains unchanged (Fig. 3 F).

On inhibition with 10^{-5}, 10^{-4}, and 10^{-3} M Physostigmine the activity of the two anodic esterases is diminished step by step,

while the β-2 esterase is greatly inhibited by 10^{-5} M Physostigmine and totally by 10^{-4} and 10^{-3} M Physostigmine.

Esterase activity following immunoelectrophoresis shows a varying picture: α-1-V has an unmistakable esterase activity, but the fraction shows changing mobility, as it may be present either as α-1 globulin or as prealbumin. Since it is not inhibited by 10^{-5} M DFP, it is assumed to be identical with the prealbumin found on agar-gel micro-electrophoresis.

After immunoelectrophoresis, moreover, α-2-III lipoprotein and the chylomicron fraction show esterase activity. This activity, however, is demonstrable only when using larger quantities of serum than usual in immunoelectrophoresis (larger application hole). These two last-mentioned esterases appear to be inhibited by 10^{-5} M DFP.

On immunoelectrophresis there is no activity in the β-2 area.

Autoradiography

α-2-I proved to have iron-binding properties (Fig. 3 H, I, J, & K) analogous to bovine β-2-I and human β-1 transferrin.

DISCUSSION

Bovine Serum

From what has been mentioned above it may be seen that a total of 17 fractions were demonstrated in bovine serum.

In the case of γ-1, β-2-II, and β-2-III the salting-out properties are almost identical, and the precipitates extend far into the anodic area, where they show confluence. They seem to make up a "γ system" analogous with the γ-β-2-A system in human serum (*Heremans, Heremans and Schultze* (10)), the γ-T system in the horse (*Wunderly* (29)), and the γ-β-3-II-β-3-III system in mice (*Clausen and Heremans* (6)). These fractions are most probably identical with the immunoglobulins of cattle. At least, immunoelectrophoresis demonstrates that the fractions found by paper and agar-gel micro-electrophoresis may be divided into immunologically different proteins having the same mobility. It must be assumed that antibody-containing globulins are present not only in the γ area, but also in the β area and as traces in in the α-2 area.

It is worth noting that the precipitate from 1.2 M ammonium sulphate apparently consists exclusively of a part of γ-1, as on

inmmunoelectrophoresis this precipitate makes a short, somewhat diffuse precipitation line, while by the present antisera it was not possible to detect other precipitation lines.

In human serum there is no precipitate analogous with γ-2, which is possibly a high-molecular protein analogous with human β-2-M. If there is one, it must have a very different mobility, as human β-2-M is so high-molecular that it can only with difficulty penetrate into the microstructure of the agar micelle. Therefore, the precipitation line for human β-2-M issues from the central application hole as a faint, almost linear band. Bovine γ-2, on the other hand, appears to penetrate unhindered into the agar micelle, so its molecular weight cannot exceed 200,000 (28).

Iron-binding β-2-I globulin manifests itself on immunoelectrophoresis of normal serum as a long line with a so-called double curvature, *i.e.* as a flat line more curved at the ends than in the centre, indicating that the precipitate has arisen as a result of a reaction between two proteins having different mobility and the same immune properties on one side and a common antibody on the other side. This means that in normal bovine serum there are presumably at least two iron-binding fractions having identical immune properties. Autoradiography shows β-2-I as an evenly curved, single line which would appear to indicate the existence of only one iron-binding fraction. In the case of human serum, however, it is known that the reaction to diluted acid at room temperature is splitting off of neuraminic acid from certain glycoproteins, including transferrin (18, 27). Splitting off of the negatively charged neuraminic acid causes a reduction in the electrophoretic mobility of the glycoprotein.

Since prior to autoradiography faintly acid ferric citrate was added to the serum, something similar might be imagined to happen to the most quickly migrating bovine transferrin fraction, its mobility being reduced to a magnitude corresponding to the mobility of the slow fraction. If so, precipitation lines of the 2 fractions would appear on immunoelectrophoresis as a single line.

As far as the β area is concerned, electrophoretic studies of bovine serum must be assessed with some reserve, as starch gel electrophoresis of normal serum, even from cattle of the same race, has shown genetic variations involving the number as well mobility of the β globulins (3, 19).

The esterase found in the γ area agar-gel micro-electrophoresis behaves like human serum cholinesterase in inhibition experiments and must, therefore, be presumed to be the serum cholinesterase of cattle. It has only faintly developed antigenic properties, since it cannot be demonstrated by immunoelectrophoresis.

PAS reaction showed that glycoproteins in cattle, in analogy with human serum, were present in the α region, especially the α-2 area. This latter area also showed faint oxidase activity which could not be established in any detail by immunoelectrophoresis.

Presumably, both α-1-III and α-1-IV are lipoproteins, but since they are present either in small quantities or else they possess little antigenic action, further determination is out of the question for the time being, as the lines shown by immunoelectrophoresis are very close to each other. The low lipid content may be due to the unvaried feed the cattle received during the time of the study. Moreover, one of the fractions just mentioned has such a marked esterase activity that owing to the pronounced blackening of the corresponding precipitate is was difficult to distinguish between the latter and the nearest fractions. The named esterase is an A esterase, as it is not inhibited by 10^{-5} M DFP.

Guinea-pig Serum

Immunoelectrophoresis affords a possibility of distinguishing between 15 fractions in guinea-pig serum. γ-1, γ-2, and β-2-I have almost identical salting-out properties, and these fractions almost coincide in immunoelectrophoresis. This indicates that immunologically too they are closely related.

Probably, these fractions make up the "γ system" of the guinea-pig.

β-2-II develops only faintly and often not at all, so this fraction must be assumed be a week antigen.

α-2-III stains easily with lipid. Its cathodic part coincides with the chylomicron fraction which also takes lipid stain. The latter fraction is not antigenic, but may be demonstrated by immunoelectrophoresis as a short, diffuse line issuing from the application hole in the direction of the anode.

α-2-I is able to bind iron analogous to human β-1-transferrin and might thus be called guinea-pig α-2-transferrin. Guinea-pig

transferrin, then, differs from bovine and human transferrin in being an α-2 globulin.

In the α-2 area, moreover, the studies showed a glycoprotein as a faint oxidase activity which could not be further elucidated.

As already mentioned, agar-gel micro-electrophoresis revealed 3 esterases in guinea-pig serum. *Goutier* (8) has described the same esterases following paper and starch gel electrophoresis and determined the prealbumin esterase as an A esterase, the α-1-α-2 esterase as B esterase, and an esterase migrating between the γ globulins as cholinesterase. The inhibition experiments following agar-gel micro-electrophoresis confirmed these results except for the finding that in the present experiments cholinesterase was found to be migrating as a β-2 globulin. The A esterase has also been described by *Aldridge* (1).

In immunoelectrophoresis the A esterase migrates either as prealbumin or as an α-1 globulin (α-1-V), which is presumably a weak antigen as it gives only a very faint precipitate, while stained for esterase activity it gives a strong reaction. Furthermore, α-1-V takes lipid stain, corresponding to the findings in agar-gel micro-electrophoresis.

The varying mobility of α-1-V shows similarities to human serum, human α-1 lipoprotein changing in character on freezing, dialysis, etc. (9, 13, 14), so that upon subsequent electrophoresis it presents itself as prealbumin. This change in mobility also occurs when it is merely left to stand at room temperature.

B esterase could not be definitely demonstrated by immunoelectrophoresis, but presumably it comprises α-2-III.

Cholinesterase could not be demonstrated by immunoelectrophoresis, so presumably it is not antigenic.

Albumin takes lipid stain after agar gel micro-electrophoresis, but this could not be confirmed by immunoelectrophoresis. However, the staining is faint. As in the the case of human albumin, this may be due to a content of acetalphosphatides (21) or fatty acids (16).

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SUMMARY

On the basis of a finding of 17 serum protein fractions in normal bovine serum and 15 fractions in normal guinea-pig serum on immunoelectrophoresis, an attempt was made to characterize the fractions in terms of salting-out properties, lipid and glycoprotein content, and esterase activity.

Parallel to the immunoelectrophoretic investigations, bovine and guinea-pig serum was subjected to agar-gel micro-electrophoresis and in one case to paper electrophoresis. Agar-gel micro-electrophoresis revealed 6—7 fractions in bovine and guinea-pig serum. In rare cases it was possible to discern further fractions in the γ area following electrophoresis of bovine serum. By means of agar-gel micro-electrophoresis the protein fractions could be characterized in more detail.

ZUSAMMENFASSUNG

Elektrophoretische Untersuchungen von normalem Kuh- und Meerschweinchenserum.

Auf Grund der durch Immunelektrophorese gefundenen 17 Serumproteinfraktionen in normalem Kuhserum sowie der 15 Fraktionen in normalem Meerschweinchenserum, wurde es versucht diese Fraktionen näher zu charakterisieren durch die Bestimmung deren Entsalzungseigenschaften, Lipoprotein- und Glykoproteingehalt sowie durch deren Esteraseaktivität.

Parallel mit den immunelektrophoretischen Untersuchungen wurden Vergleichsuntersuchungen des Kuh- und Meerschweinchenserums mittels Agargelmikroelektrophorese und in einem Falle mittels Papierelektrophorese gemacht.

Im Kuh- und Meerschweinchenserum zeigt die Agargelmikroelektrophorese 6—7 Fraktionen, in seltenen Fällen konnte man nach der Elektrophorese des Kuhserums im γ-Gebiet mehrere Fraktionen unterscheiden. Mittels die Agargelmikroelektrophorese war es möglich die Proteinfraktionen näher zu charakterisieren.

SAMMENDRAG

Elektroforetiske undersøgelser af normalt kvæg- og marsvineserum.

På grundlag af fundet af 17 serumproteinfraktioner i normalt kvægserum samt 15 fraktioner i normalt marsvineserum ved immunelektroforese søgtes de fundne fraktioner nærmere karakteriseret ved deres udsaltningsforhold, lipoid- og glycoproteinindhold samt esteraseaktivitet.

Parallelt løbende med de immunelektroforetiske undersøgelser underkastedes kvæg- og marsvineserum agargelmikroelektroforese og i et enkelt tilfælde papirelektroforese. Agargelmikroelektroforese viser 6—7 fraktioner i kvæg- og marsvineserum, i sjældnere tilfælde kunne der i γ-området efter elektroforese af kvægserum skelnes flere fraktioner. Agargelmikroelektroforesen muliggjorde yderligere karakterisation af de fundne proteinfraktioner.

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