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IMMUNOLOGIC STUDIES ON PALE SOFT EXUDATIVE MUSCLE IN PIGS

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РЕЗЮМЕ

Проведен сравнительный иммунохимический анализ растворимого мышечного протеина бледной эксудативной мягкой мускулатуры /БЕМ/ и нормальной мускулатуры/м. Лонгиссимус дорси/ свиней.Иммунные сыворотки против мускульных экстрактов были получены из двух групп зайцев/в каждой группе по 6 штук/.

Результаты двойной иммунодифузии и иммуноэлектрофорезы показывают более значительную преципитационную активность гомологной и гетерологной антисыворотки по отношению антигена-экстракта бледной эксудативной мускулатурой /БЕМ/.Устанавливается наличность антигенного несоответствия между бледной эксудативной мускулатурой /БЕМ/ и нормальной мускульной тканей.Являются различия в числе,позиции и характере преципитационных систем, полученные при бледной мягкой эксудативной мускулатура/БЕМ/ с гетерологной и аналогной антисыворотки по сравнению с нормальной мускулатурой.Демонстрированные изменения в иммуногенной специфичности мускульного протеина бледной мягкой эксудативной мускулатуры,объясняется возможностью его молекулы терпеть конформационные изменения/касающиеся высших структур протеиновой молекулы/ при резко изменяющихся условиях бледной мягкий эксудативной мускулатуры/БЕМ/.

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On a effectué une analyse comparative immunochimique des protéines musculaires solubles d'une musculature exsudative pâle et tendre (MEP) et d'une musculature normale (m.Longissimus dorsi) chez les porcins.

Les immunoserums contre les extraits musculaires ont été obtenus de deux groupes de lapins (chacun consistant en six animaux).

Les résultats de l'immunodiffusion double et de l'immunoélectrophorèse démontrent une activité plus significative de précipitation des antisérums homologue et hétérologue envers l'antigène - extrait d'une musculature exsudative pâle

(MEP). On établit la présence d'une disparité antigène de la musculature exsudative pâle (MEP) par rapport au tissu musculaire normal. On peut apercevoir des différences de nombre, de position et de caractère des systèmes de précipitation, obtenus avec une musculature exsudative pâle et tendre (MEP) avec les antisérums hétérologue et analogue par rapport à la musculature normale.

La modification, manifestée dans la spécificité immunogène des protéines musculaires de la musculature exsudative pâle et tendre (MEP), peut être expliquée par la capacité de la molécule de pouvoir subir des changements de conformation (concernant les structures supérieures de la molécule protéique) lors des modifications drastiques de la musculature exsudative pâle et tendre (MEP).

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A comparative immunochemical analysis was made of the soluble muscle proteins from pale soft exudative (PEM) and normal muscle (m.Longissimus dorsi) of pigs.

Immune sera against muscle extracts were obtained from two groups of rabbits (each consisting of six).

The results from the double immunodiffusion and the immunoelectrophoresis indicate a more considerable precipitating activity of the homologous and heterologous antisera towards the antigen - an extract of PEM muscle. The existence of antigenic discrepancy between PEM muscle and normal muscle tissue is established. There are differences in the number, position and character of the precipitation systems obtained with PEM muscle with the heterologous and analogous antisera in comparison with normal tissue.

The change demonstrated in the immunogenic specificity of muscle proteins from PEM muscle is explained with the possibility for their molecule to undergo conformation changes (concerning the higher structures of the protein molecule) under the drastically altered conditions of PEM muscle.

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Es wurde eine vergleichende immunologische Analyse der löslichen Muskelproteine einer blassen exsudativen wei-

chen Muskulatur (BEM) und einer normalen Muskulatur (vom Musc.longissimus dorsi) von Schweinen durchgeführt.

Die Antiseren der Muskelextrakte wurden von zwei Gruppen Kaninchen (je 6 Stück in einer Gruppe) erhalten.

Die Ergebnisse von der doppelten Immundiffusion und von der Immunelektrophorese zeigen eine erhebliche Präzipitationsaktivität sowohl der homologischen, als auch des heterologischen Antiserums gegen das Antigen - ein Extrakt aus einer blassen exsudativen Muskulatur (BEM). Es wird das Vorhandensein einer antigenen Nichtübereinstimmung zwischen der blassen exsudativen Muskulatur (BEM) und dem normalen Muskelgewebe festgestellt. Im Vergleich zur normalen Muskulatur bestehen Unterschiede in der Zahl, in der Position und im Charakter der Präzipitationssysteme, welche aus der blassen exsudativen Muskulatur (BEM) durch das heterologische und analogische Antiserum erhalten sind.

Die zum Vorschein gekommenen Veränderungen in der immunogenen Spezifität der Muskelproteine der blassen weichen exsudativen Muskulatur (BEM) ist mit der Möglichkeit zu erklären, dass bei den drastish veränderten Bedingungen der blassen weichen exsudativen Muskulatur (BEM) im Molekül Konformationsveränderungen (welche die höheren Strukturen der Proteinmoleküle betreffen) eintreten.

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The ethiopathogenesis of pale exudative muscles (PEM) in pigs is still an unsolved problem. For the occurance of PEM are pointed many factors - genetic (Ludwigsen 1958, Sayre 1963, Bickhardt 1971, Judge 1971), technological (Sibesma 1971, Lawrie 1971) and others (Sayre 1963, Forest 1963).

During the last dekade, center of the attention of research workers remains the essence of the processes envolved with PEM. Ludvigsen(1954), Topel et al. (1968), Walstra (1971), Sibesma (1970) and others,point as a dominant initial moment an unbalance of chormonal regulation during the stress reaction.

Biochemical investigations demonstrate, that with PEM the glycolitic processes are distorted as well as the activity of the enzymes phosphorilase, adenosinthreephosphatase, lactatdehydrogenase, kreatinphosphokinase and others (Saye 1963, Kolberg 1963, Bendal et al. 1963, Briskey & Wismer-Pedersen 1961, Hamm 1969, Sibesma 1971). Glycolises flows very fast, based on intensive decomposition of ATP and others phosphonucleotides, as a result of which, the increased concent ration of lactic acid leads to a strong decrease of pH (Hamm and Potthast 1972, Lister 1970). Related to these processes quite significant is the condition of the muscle proteins. McLaughlin 1963, Sayre 1964 and others, report that the solubility of the muscle proteins decrease, Scopes and Lawrie (1963) report, that sarcoplasma proteins denaturate and precipitate upon the miofibriles, which fact accounts for the change in their solubility. As most characteristic indice for PEM, Bendal and Lawrie (1964) point the lowered extractibility of proteins in solution on high or low ionic strenght.

Inspite of the many biochemical investigations of PEM, the changes in the properties of muscle proteins are limited only to a determination of the degree of solubility, extractibility in solutions of different ionic strenght, degree of precipitation (turbidity) of the solutions at different temperatures, and pH values of the media. Bendal & Wismer-Pedersen (1962), McLaughlin (1963), Scope & Lawrie (1963) McClein (1969).

To this moment, in the literature there are no discussions and evidently there are no investigations on the nature of the changes occuring in the immunogenic properties of muscle proteins on the bases of the appearance of nonspecific structures in connection with the drastic changes envolved with PEM.

Material and Methodics

Antisera were obtained from two groupes, each consisting of 6 clinically healthy rabbits with one of the following antigens:

a) extract of the soluble muscle proteins from normal tissue from M. long. dorsi.

b) extract from the soluble muscle proteins from muscle tissue of M. long. dorsi with clearly expressed signs of PEM.

The extracts were obtained as follows: samples each of 10 g from normal and pathologically changed muscles were submitted to homogenisation in an ice bath and quarz sand in physiological solution 1 : 1-W/V. The homogenate was obtained by centrifugation for 20 minutes at 1°C and 10.000 r/min. The clear supernatant was placed in ampules and stored at 2°C to the moment of immunisation.

Immunisation was made on 6 rabbits with extract of normal tissue, and on 6 rabbits with extract of PEM after the following pattern:

During the first day of immunisation, each rabbit received 1 ml (from the respective for the group extract, included in 1 ml of Freunds (Diphco) adjuvant, applied intracutaneously on 8 places of the nuchal region. After 7 days, 1 ml antigen was applied intradermally with 1 ml of not full Freunds adjuvant. Three days later were made four innoculations, each one within three days of the first: the first and second intravenously with 0,5 antigen, the third subcutaneously with 1 ml and the fourth intravenously with 1 ml antigen. After an interval of 5 days were made two consecutive innoculations in the muscles, within three days one from the other with 0,5 ml antigen. After conclusion of the immunisation, the recepients showing acceptable titres of the antibodies were blooded. The examination of the titres was made after the ring precipitation method of Martin (1943).

For determining the immune specifity of the two tested antigens was used the method of double immunodiffusion in agar gel after Ouchterlony. Difco agar 1,5% was used with 1 : 1000 Merthyolate as conservant. Diffusion was carried out at room temperature for 48 hours in a moist chamber. After the immunodiffusion was completed, the agar plates were washed with saline solution, and then stained with Amidoschwarz 10 B.

Immunoelectrophoresis was carried out after the method of Grabar-Scheidiger (1960) in 1% agar gel in veronal-medinal

against normal muscle tissue). The character of some separate immunoprecipitation lines is also changed to a certain extend.

The extract of pale exudative muscles produces three more precipitation lines with the analogue antiserum in the zone of alpha and beta globulines and three more precipitation lines with the heterologic antiserum, in comparison with those from the extract from normal muscle tissue, which are in the zone of the beta globulines.

To a certain extend is changed also the electrophoretic mobility of the proteins from the pale exudative muscles, as evidenced from figure 4, obtained during the first hours of the immunodiffusion after electrophoresis of the antigens.

Like the immunoelectrophoresis, with double immunodiffusion is demonstrated the more significant precipitation activity of antisera obtained against normal muscle tissue, in comparison to the heterologic antigen-extract from pale exudative muscles. Both methods prove the existence of antigen components of pale exudative muscles, which are not common or close by structure with the antigens of normal muscle tissue. Existing is a difference in number, position, and character of the precipitation systems obtained from pale exudative muscles with the heterologic and analogic antiserum in comparison with normal muscle extract.

Discussion

The results obtained reveal, that as a result of the development of pale exudative muscles a new condition is obtained in a part of the muscle proteins, as the existence of more clearly defined immunogenic systems from the pale exudative muscles is proved. On the one hand it could be presumed that following the strong impact on muscle metabolism in pale exudative muscles are reached limits near those of an inflamatory process or in the best case, to a distorted passage of blood vessels, so that the additional precipitation systems obtained from both immunologic methods may be due to the existence of serum proteins in the pale exudative muscles as a result of diapedesis.

buffer of pH = 8,6. The wells for the antigen measured 3 mm in diameter, and the trenches 3 mm in width and 6 cm in lenghh. Antigens were devided in an electric field for 1 hour and 250 V, while immediately after the electrophoresis was completed, the antisera were added to diffundate for 20 hours.

Results

Results obtained by the double immunodiffusion in agar gel after Ouchterlony, are quite demonstrative in relation to the antigen inconsistency (partial similarity) between the extracts from normal muscle tissue and pale exudative muscles from M. l. dorsi (fig. 1). As the figure shows, against the wells with normal muscle extract are obtained two precipitation lines with the respective antiserum and five precipitation lines against the wells with the extract of pale exudative muscles. Between the two antigens exists antigennic identity only on the nearest precipitation line, next to the central well. The second line obtained against the pale exudative muscles has a partial similarity to the lines obtained with the wells from normal muscle extract. The remaining four precipitation arcs; obtained against the pale exudative muscles, have no analogues with the wells with normal muscle extract. This indicates that in the pale exudative muscles has occured a change in the antigen composition of the muscle tissue, expressed with the existence of new antigen structures.

From the immunoelectrophoretic analyses (fig. 2 and 3) it is evident, that the extract from normal muscle tissue with the respective antiserum produces seven precipitation systems, while with the antiserum against the pale exudative muscles they are only four. Lacking are the immunoprecipitations between the extract from normal muscle tissue and the antiserum against the pale exudative muscles in the albumin zone.

Towards the antigen obtained from pale exudative muscles is developped a richer spectrum of immunoprecipitation systems, similar with the analogue antiserum (antiserum against pale exudative muscles) as well as with the heterologic (antiserum

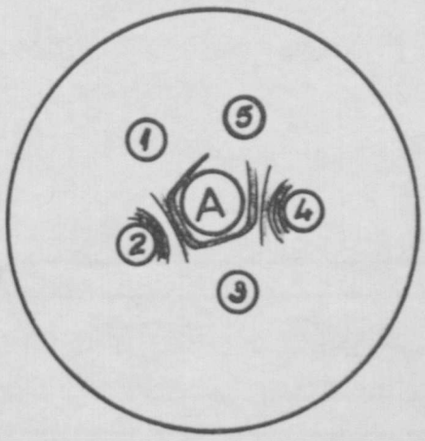


Figure 1

Double diffusion in agar gel. Central well:
A, antiserum against muscle extract from normal muscles
1 and 3 - muscle extract from normal muscles
2 and 4 - muscle extract from pale exudative muscles
5 - physiologic solution (control)

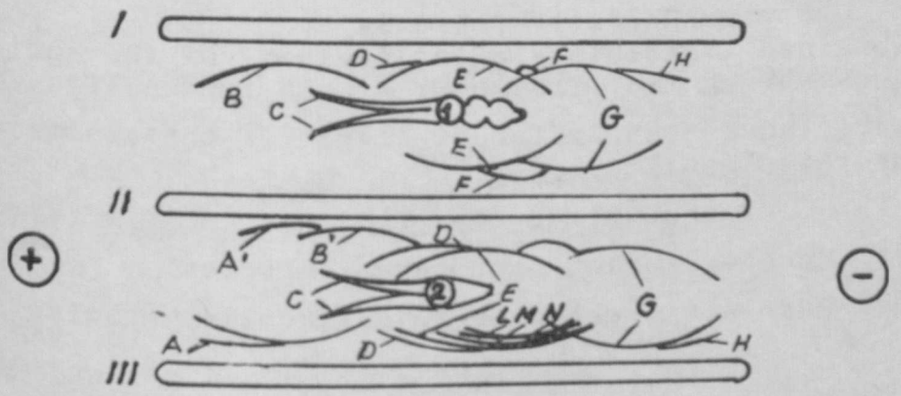


Figure 2

Immunophoresis

Round wells:
1 - Muscle extract from normal muscles
2 - Muscle extract of pale exudative muscles

Longitudinal wells:

I and III - antiserum against muscle extract from normal muscles

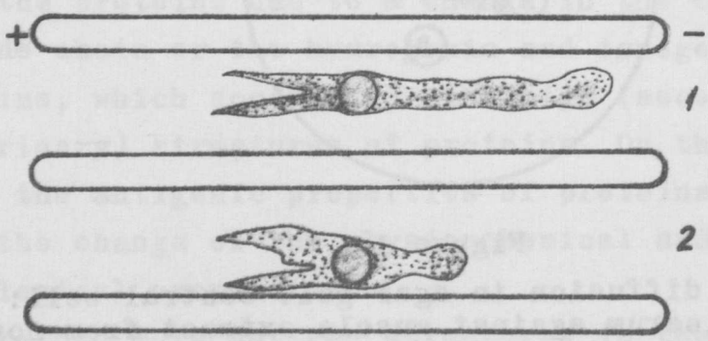
II - antiserum against muscle extract from pale exudative muscles

More probable, and acceptable is, that the change in the immunologic properties of muscle proteins proved with this experiment with pale exudative muscles, are connected with the property of their molecule to endure conformation changes under the drastically changed conditions existing in pale exudative muscles. Under these conditions, could not be excluded a denaturation of the proteins due to a change in the covalent bonds of the peptide chain or the hydrogenic and ionogenic bonds of the side chains, which account for the high (secondary, terciary and quandrinary) structures of proteins. On this basis are also changed the antigenic properties of proteins which is connected with the change of the physicochemical and further with their technological properties.

The obtained more antigen structures in the experiment with pale exudative muscles, and the changed electrophoretic mobility, could be also connected with the dissociation of some of the above-molecular structures to subunits. This denaturation of some proteins is in close dependance with pH ionic strength and the change of temperature (Jolly), which factors are also realized in the pale exudative muscles.

It should be noted that the precipitation arcs obtained by both immunochemical methods demonstrate the minimal possible antigen-titel systems, as under the conditions of the test the realization of unsoluble complexes is also possible, while in pale exudative muscles, these complexes are more than with normal muscle extract and which have possibly not developped. Only with this, could be explained the smaller number of immunoprecipitation lines of normal muscle extract with the heterologic and analogic antisera.

The proved by the experiment change in the immunogenic specificity of muscle proteins from pale exudative muscles could explain part of the many processes which take part in this pathologic phenomenon. This approach to the investigation, not taken up by other authors, could elucidate the pathogenesis of pale exudative muscles, taking into considerations the present possibilities of immune reactions which could be effectively applied as a test to proving the development of pale exudative muscles in animals.



Figures 3

Immunoelectrophoresis
obtained immediately after deviding of the antigens during the first hour of the immunodiffusion

Revealed is the different degree of the electrophoretic mobility.

Round wells:

1 - Muscle extract from normal muscles

2 - Muscle extract from pale exudative muscles

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