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The Reaction of Heart and Other Organ Extracts with the Sera of Animals Immunized with Group A Streptococci

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Summary. Extracts of guinea-pig skeletal muscle, heart, kidney and liver tissues contain soluble antigens which react in gel precipitation and immunoelectrophoresis with sera of animals immunized with group A streptococci, and with γ-globulin preparations obtained from these sera. These are specific immunological reactions. The antigens in extracts of heart tissue and other organs are proteins. They are cross-reactive antigens since absorption of sera with homologous strains of streptococci abolishes these reactions.

INTRODUCTION

Common or so-called cross-reactive (CR) antigens between micro-organisms and mammalian tissues may be significant in causing autoimmune reactions and determining the virulence of micro-organisms.

The role of CR antigen in autoimmune reactions was first substantiated by Kaplan and co-workers (Kaplan and Meyeserian, 1962a, b; Kaplan, 1963, 1965; Kaplan and Suchy, 1964). In these investigations CR antigen was discovered in the cell-walls of group A streptococcus strains, and antibodies to this antigen reacted with sarcolemma of myofibres of the myocardium and skeletal muscles of animals and man as was shown by immunofluorescence. Anti-streptococcal sera also reacted with the smooth muscle of vessels in the myocardium. The same findings were reported by Zabriskie, Freimer and Seegal (1964) and Zabriskie and Freimer (1966). In their opinion the CR antigen is present in the cytoplasmic membrane of most strains of group A streptococci.

Common antigens were also found by other workers between micro-organisms and human and animal kidney, colon, heart and skin (Markowitz and Lange, 1964; Chase and Rapaport, 1965; Perlmann, Hammarstrom, Lagercrantz and Gustafsson, 1965; Mazina and Rassokhina, 1966).

In our previous studies complement fixing antibodies to extracts of heart were found in the sera of animals immunized with cultures of group A streptococci (Lyampert, Beletskaya, Borodiyuk and Smirnova, 1962). It was shown by immunofluorescence that sera of rabbits immunized with group A streptococci, types 1, 5 and 29 react with different elements of the myocardium and skeletal muscles of animals and man (Lyampert, Grizlova, Beletskaya and Danilova, 1964; Lyampert, Danilova and Borodiyuk, 1966a;

Lyampert, Danilova, Borodiyuk and Beletskaya, 1966b; Lyampert, Vvedenskaya and Danilova, 1966c; Lyampert, Borodiyuk, Vvedenskaya and Danilova, 1966d; Danilova, 1966). Absorption with homologous strains of streptococci abolished these reactions. The presence of four common antigens was demonstrated in cultures of group A streptococci, types 1, 5 and 29, and in the heart. It has been established that antibodies to CR antigen should be considered as autoantibodies since they react with organ-specific tissue antigens (Lyampert *et al.*, 1966c, d).

Some workers have suggested a possible role of common antigens in determining the virulence of micro-organisms (Rowley and Jenkin, 1962; Mekler, 1965).

The biological (immunological) action of the CR antigens of micro-organisms appears to depend on two factors: (1) what antigens (isoantigens or organ-specific antigens) they share with the host; and (2) whether there is tolerance to these substances (Lyampert *et al.*, 1966 c, d).

To investigate the mechanism of autoimmune reactions it would be valuable to know more about the chemistry of common antigens.

The common antigens of type 12 streptococci, and kidney basement membranes were found to be soluble glycoproteins (Marcowitz and Lange, 1964). The CR antigens of streptococci and heart tissue were shown to be proteins (Kaplan, 1963; Lyampert *et al.*, 1966a). In the above mentioned works the common antigens in the heart tissue were investigated by complement fixation and immunofluorescence.

In the present study, antigens were found in extracts of heart, skeletal muscle, liver and kidney tissue by precipitation reactions and by immunoelectrophoresis in gel with the sera of animals immunized with cultures of group A streptococci. Experiments using absorption with streptococcal cultures showed that these substances could be considered as CR antigens. In the course of this work non-immunological precipitation reactions were observed (Kidd and Friedewald, 1942; Boyden, 1966), and investigations were undertaken to differentiate true immunological reactions from non-immunological ones.

MATERIALS AND METHODS

Strains

Cultures of group A streptococci, type 1 (No. 2/55), type 5 (No. 6/55) and type 29 (No. 15/15) (Prague collection) containing M proteins underwent six to ten passages in casein medium with yeast extract and were harvested from it.

Sera

Rabbits were immunized first with killed and then with living cultures according to the procedure described earlier (Lyampert *et al.*, 1966a, c). The blood was collected after the fourth, fifth and sixth courses of immunization. Sera of non-immunized rabbits as well as sera obtained prior to immunization served as controls.

Absorption of sera with streptococcal cultures

Strains repeatedly passed in the above mentioned medium were grown for 24 hours in it and the organisms were recovered and washed three times with saline. Solid sediment of the culture containing 10^9 microbial cells was added to each millilitre of the sera to be absorbed. The mixture was incubated for 2 hours at 37° and overnight at 4°.

Tissue extracts

Extracts from guinea-pig and rabbit heart and guinea-pig skeletal muscles, liver, kidney and spleen tissues were made. Pieces of tissue, both fresh and frozen at −20°, were washed and homogenized in saline, buffered with 0·01 M phosphate, pH 7·2, using a tissue homogenizer; 4·0 ml of buffer was used for each gram of the tissue. Extraction lasted 48 hours, with shaking of the suspension for the first 6–12 hours. The suspension was centrifuged for 1 hour at 6000 rev/min. All procedures were performed at 4°. The extracts were concentrated ten-fold by means of evaporation under the fan, dialysed against medinal-veronal buffer, pH 8·6, and centrifuged at 9000 rev/min for 1 hour. For preservation, penicillin and streptomycin were added, 100 units of each per 1 ml of extract. The extracts were kept frozen at −20°. Solutions of penicillin and streptomycin (up to 1000 units/ml each) and the nutrient medium alone were used as controls. In some experiments normal guinea-pig serum was used as antigen.

Sedimentation of tissue antigens

Guinea-pig heart tissue extract was centrifuged in a Spinco ultracentrifuge at 30,000 ***g*** for 30 minutes, and at 110,000 ***g*** for 1 hour.

Isolation of γ-globulin and albumin from the sera

Albumin was isolated from immune rabbit sera by means of preparative electrophoresis in gel (Grabar's method as modified by Zilber and Abelev, 1962). γ-Globulins from immune and normal sera were prepared (Baumstark, Laffin and Bardawil, 1964) on DEAE Sephadex A-50 (coarse, in chloride form) by means of repeated chromatography at pH 6·5 and were then lyophilized. The preparations obtained were tested for purity in immunoelectrophoresis against donkey anti-rabbit serum.

Gel precipitation

The tissue extracts were tested against immune and normal sera and preparations of γ-globulin or albumin according to the method of Ouchterlony in a micromodification suggested by Gusev and Tsvetkov (1961).

Immunoelectrophoresis in gel (IEP)

This was carried out according to the method of Grabar as modified by Zilber and Abelev (1962) at 6 V/cm and 25 mA for 1 hour. Pyronine and albumin, moved 2 and 1·2 cm, respectively, away from the origin in this time.

In these experiments, preparations of γ-globulin were used at a concentration of 100 mg/ml and of albumin at a concentration of 300 mg/ml. In some cases, after the normal and immune sera had been placed in wells and exposed to electrophoresis, the troughs were filled with tissue extracts. The results of gel precipitation and IEP were read at 24 or 48 hours.

RESULTS

1. REACTIONS OF HEART TISSUE EXTRACTS WITH ANTI-STREPTOCOCCAL SERA

Sera of animals immunized with group A streptococci, type 1, were found to produce distinct precipitin lines in three out of twelve cases on testing in gel precipitation against

guinea-pig heart tissue extract. The latter also reacted positively with four out of eight sera prepared against type 29 streptococci and gave a slight positive reaction with two out of nine antisera against type 5. The most pronounced reactions were with sera obtained after the fifth and sixth cycles of immunization. Tests using several different extracts all showed positive reactions with the same sera. The guinea-pig heart-tissue extracts did not react with forty-nine normal rabbit sera nor with ten sera obtained from animals before immunization. Seven of these ten pre-immunization sera were obtained from rabbits which developed antibodies after immunization.

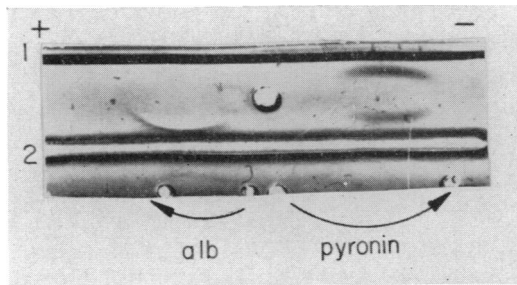

FIG. 1. Immunoelectrophoresis. Reaction of immune sera with heart tissue extracts. Well: Rabbit serum against type 29. Troughs: (1) Guinea-pig heart tissue extract, (2) rabbit heart tissue extract.

The extracts of rabbit heart tissues gave wide diffuse precipitin lines with almost all anti-streptococcal and normal rabbit sera, but in some cases gave two precipitin lines with immune sera. Immunoelectrophoresis was employed to show whether the extracts of heart tissues were reacting with immunoglobulins in the sera (Fig. 1). The immune sera were found to react positively with guinea-pig heart tissue extracts only at the site of pyronine localization, i.e. in the γ-globulin zone. Rabbit heart tissue extract reacted with the immune sera both in γ-globulin and albumin zones. Normal sera did not react with guinea-pig heart tissue extracts but showed reactions with rabbit heart tissue extract in the albumin zone.

The γ-globulin preparations obtained from immune sera against different types of streptococci and from normal rabbit sera on immunoelectrophoresis with the donkey anti-rabbit sera contained γ-globulins and some β-globulins (Fig. 2). The extracts of

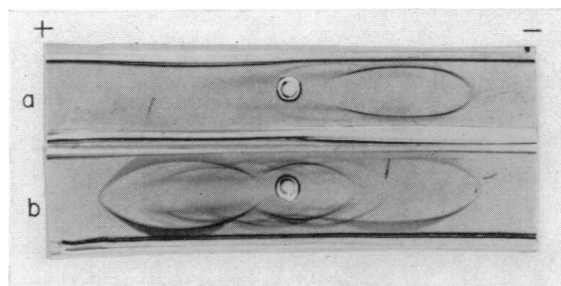

FIG. 2. Immunoelectrophoresis. Reaction typical for the γ-globulin preparations. Wells: (a) γ-globulin fraction, (b) whole rabbit serum. Troughs: Donkey anti-rabbit serum.

heart tissues of guinea-pigs and rabbits were tested with the preparations of γ-globulin by gel precipitation (Fig. 3B). The extracts of guinea-pig heart tissues produced distinct reactions with γ-globulins obtained from the antisera against types 1 and 29, and a slightly positive reaction with γ-globulin prepared from the serum, type 5. Negative results were obtained in controls testing the immune sera against the antibiotics and nutrient medium.

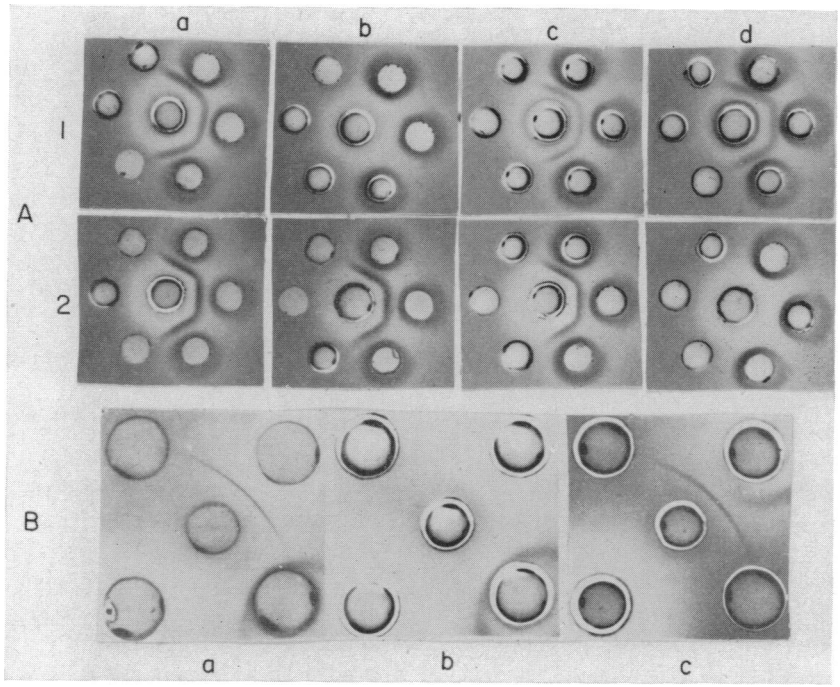

FIG. 3. Gel precipitation reactions of anti-streptococcal sera and a γ-globulin preparation with the guinea-pig heart tissue extract before and after absorption with streptococcal cultures. (A) Central wells: (1) antiserum against type 1, (2) antiserum against type 29 (a) non-absorbed, (b) absorbed with type 1 culture, (c) absorbed with type 5 culture, (d) absorbed with type 29 culture. In the wells at the periphery (to the right) guinea-pig heart tissue extract (non-diluted), (to the left) buffer. (B) Central wells: γ-globulin preparation from type 1 serum. (a) Non-absorbed, (b) absorbed with type 1 culture, (c) absorbed with type 29 culture. In the top wells at the periphery (to the right) guinea-pig heart tissue extract.

Absorption with the cultures of types 1, 5 and 29, was carried out to elucidate whether the formation of antibodies, reactive with heart tissue antigens, was due to the presence of common antigens in the streptococcal cultures. In these experiments, the sera, absorbed and non-absorbed with the streptococcal cultures, were tested in gel precipitation with the guinea-pig heart tissue extracts. Similar experiments were performed with the preparations of γ-globulin obtained from the type 1 and type 29 antisera (Fig. 3). It was possible to abolish the reaction with an extract of heart tissue only when the serum or γ-globulin was absorbed with the same strain as was used for immunization. Cultures of other type did not absorb antibodies reactive with the heart tissue extracts. The antigens reacting with the sera types 29 and 1 appeared to possess different electrophoretic mobility (Fig. 4).

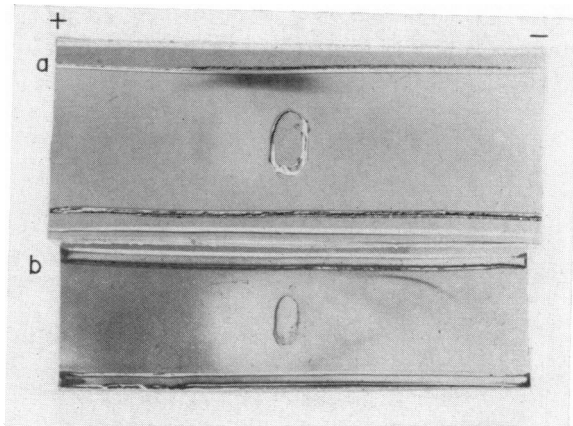

FIG. 4. Immunoelectrophoresis of the guinea-pig heart tissue extract demonstrating the existence of CR antigens with different electrophoretic mobility. Troughs: (a) γ-globulin from type 29 serum, (b) γ-globulin from type 1 serum. Wells: guinea-pig heart tissue extracts.

Heart tissue antigens reactive with type 1 and type 29 antisera are apparently proteins, since trypsin-treated extracts did not react with these sera. These substances are most likely soluble components of tissues since after centrifugation at 30,000 and 110,000 ***g*** the supernatant was still able to react with γ-globulin obtained from type 1 serum, and whole antisera against types 1 and 29.

2. THE REACTION OF EXTRACTS OF SKELETAL MUSCLE, KIDNEY AND LIVER TISSUES WITH ANTI-STREPTOCOCCAL SERA

Anti-streptococcal and normal rabbit sera were tested in gel precipitation with extracts of guinea-pig skeletal muscle, kidney, liver and spleen tissues. Distinct precipitation reactions were obtained with extracts of skeletal muscle, with the same type 1 and 29 antisera as reacted with extracts of guinea-pig heart tissue. Thirty-eight normal rabbit sera failed to react with extracts of skeletal muscle. Liver and kidney tissue extracts reacted not only with immune sera but with most normal sera as well. Guinea-pig serum used as antigen failed to react with both normal and immune rabbit sera.

To exclude non-immunological reactions, extracts of skeletal muscle, liver and kidney tissues were tested in immunoelectrophoresis. The sera were placed in wells, and after electrophoresis the troughs were filled with skeletal muscle, liver and kidney tissue extracts. Extracts of guinea-pig skeletal muscle tissues reacted with immune sera only in the γ-globulin zone and did not react with the normal sera. Extracts of kidney and liver tissues, similar to those of rabbit heart tissue, reacted with type 29 antiserum, both in the zone of γ-globulin and in the zone of serum albumin. Normal sera reacted with extracts of liver and kidney tissues only in the zone of serum albumin (Fig. 5).

Antigens in guinea-pig skeletal muscle and heart tissue reacted with γ-globulin obtained from sera against type 1 streptococci, giving a reaction of identity (Fig. 6). Extracts of other tissues did not react with this γ-globulin. The reaction with skeletal muscle and

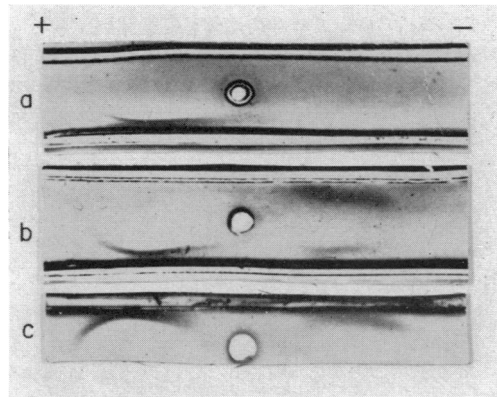

FIG. 5. Reactions of normal and anti-streptococcal rabbit sera with extracts of guinea-pig tissues in immunoelectrophoresis. (a) Well: normal rabbit serum; top trough, extract of skeletal muscles; bottom trough, extract of liver. (b) Well: anti-type 29 antiserum; top trough, extract of skeletal muscles; bottom trough, extract of kidneys. (c) Well: anti-type 29 antiserum; trough, extract of liver.

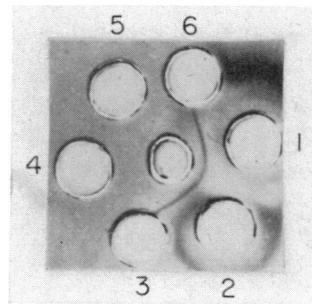

FIG. 6. The reaction of antistreptococcal type 1 immune γ-globulin with guinea-pig tissues extracts. Central well: γ-globulin obtained from type 1 antiserum. Peripheral wells containing tissue extracts: (1) skeletal muscle; (2) heart; (3) liver; (4) kidney; (5) spleen. Well 6: buffer.

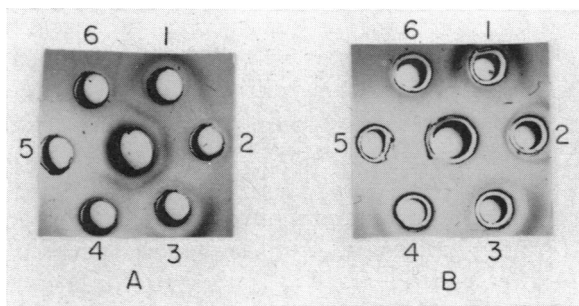

FIG. 7. The reaction of type 29 immune γ-globulin with guinea-pig tissues extracts. Central wells: γ-globulin. (A) Non-absorbed, (B) absorbed with type 29 culture. Peripheral wells with tissue extracts: (1) skeletal muscles; (2) heart; (3) liver; (4) kidney; (5) spleen. Well 6: buffer.

heart tissue was abolished by absorption with the homologous but not with the heterologous strains. The γ-globulin obtained from type 29 antisera gave precipitation in gel against antigens in skeletal muscle, liver and kidney tissue extracts. These antigens gave reactions of identiity with each other as well as with the heart tissue antigen reactive with the same γ-globulin. These reactions could be abolished by the absorption with the homologous type of streptococci (Fig. 7).

Extracts of guinea-pig liver and kidney tissues reacted with albumin obtained from immune rabbit sera by preparative electrophoresis. Attempts to abolish these reactions by absorption with streptococcal cultures were unsuccessful.

DISCUSSION

The present study gives evidence of soluble antigens in guinea-pig skeletal muscle, heart, liver and kidney tissue extracts which react with the sera of animals immunized with streptococci. Immunoelectrophoresis and preparation of γ-globulin showed that one of the serum reactants was an immunoglobulin. In addition, rabbit heart tissue extracts give 'spurious' reactions with serum albumin. The 'spurious' reactions were also demonstrated with guinea-pig liver and kidney tissue extracts, but not with guinea-pig muscle and heart tissue extracts.

Some workers (Kidd and Friedewald, 1942; Tomasi, 1961; Boyden, 1966) consider that components of normal sera reacting with organ extracts are either α_2-globulins or albumins. Serum component(s) possessing the electrophoretic mobility of serum albumin have been also reported to react with synthetic polypeptides (Maurer, Gerulat and Pinchuck, 1964). In our studies, the extracts of tissues that produced the 'spurious' reactions with albumin of rabbit serum also reacted with crystalline bovine albumin and highly purified human albumin. Stronger reactions were observed with sera from immunized animals, as compared with normal ones. We did not succeed in abolishing the reactions with albumin by absorption with streptococcal cultures.

The antigens of heart tissue that reacted with immunoglobulins of sera from animals immunized with different types of streptococci are apparently different. The evidence for this is the cross-absorption performed with different types of streptococci and the different electrophoretic mobility of antigens reacting with the antisera to types 1 and 29. One of the antigens in skeletal muscle extracts is similar to an antigen of heart tissue which reacts with type 1 antiserum. Another antigen of skeletal muscle tissue extracts, which reacts with type 29 antiserum, is probably similar to one of the antigens found in heart, liver and kidney extracts. The results of absorbing antisera with streptococcal strains are evidence of corresponding CR antigens in the cultures of types 1 and 29 streptococci. Antigens of heart tissue seem to be proteins since they can be destroyed by trypsin. More investigations are needed to decide whether these common antigens correspond to those described previously (Kaplan *et al.*, 1962a, b; Kaplan, 1963, 1964, 1965; Marcowitz *et al.*, 1964; Lyampert *et al.*, 1964, 1966a, b, c, d).

The sera used in the present study contained antibodies to the same components of heart tissue as detected by the indirect immunofluorescence method and reported earlier (Lyampert *et al.*, 1966c). Some of the sera also reacted with kidney and liver by immunofluorescence (Danilova, 1967).

The mechanism of development of autoimmune reactions due to immunization with CR antigens seems to depend on the nature of the common antigen. If it is a protein

bearing not only common but foreign determinants as well, 'termination' of tolerance may take place (Weigle, 1963, 1965). Autoimmune reactions can also occur when the common protein antigen corresponds to the so-called 'hidden' determinants or 'sequestrated' antigens to which there is no tolerance. If the CR antigen is a hapten it can acquire immunizing effect as a result of conjugation with a foreign protein of the micro-organism. It may be that in this case the 'termination' of tolerance is also involved since it has been suggested (Glynn, Holborow and Johnson, 1956; Glynn and Holborow, 1965) that tolerance may exist to some haptens.

REFERENCES

BAUMSTARK, J. S., LAFFIN, R. J. and BARDAWIL, W. A. (1964). 'A preparative method for the separation of 7S gamma globulin from human serum.' *Arch. Biochem. Biophys.*, **108**, 512.

BOYDEN, S. V. (1966). 'Natural antibodies and the immune response.' *Advanc. Immunol.*, **5**, 1.

CHASE, R. M. and RAPAPORT, F. T. (1965). 'The bacterial induction of homograft sensitivity. I. Effects of sensibilization with group A streptococci.' *J. exp. Med.*, **122**, 721.

DANILOVA, T. A. (1966). 'Investigation of common antigen of group A streptococci and heart tissue of man and experimental animals by immunofluorescent method. (In Russian). *J. Microbiol. Epidemol. Immunobiol.*, **2**, 94.

DANILOVA, T. A. (1967). 'Reaction of antistreptococcal sera with some elements of guinea-pig tissues by immunofluorescent method.' (In Russian). *Bull. exp. Biol. Med.*, **5**, 70.

GLYNN, L. E., HOLBOROW, E. J. and JOHNSON, G. D. (1956). 'The influence of the A-like substance of rabbits on their immune response to human group-A substance.' *J. Immunol.*, **76**, 390.

GLYNN, L. E. and HOLBOROW, E. J. (1965). *Autoimmunity and Disease*. Blackwell Scientific Publications, Oxford.

GUSEV, A. I. and TSVETKOV, V. S. (1961). 'The technique of the reaction of microprecipitation in gel.' (In Russian). *Lab. Delo*, **2**, 43.

KAPLAN, M. H. and MEYESERIAN, M. (1962a). 'An immunological cross reaction between group A streptococcal cells and human heart tissue.' *Lancet*, **i**, 706.

KAPLAN, M. H. and MEYESERIAN, M. (1962b). 'Immunological studies of heart tissue. V. Antigens related to heart tissue revealed by cross-reaction of rabbit antisera to heterologous heart.' *J. Immunol.*, **86**, 450.

KAPLAN, M. H. (1963). 'Immunological relation of streptococcal and tissue antigens. I. Properties of an antigen in certain strains of group A streptococci exhibiting an immunologic cross-reaction with human heart tissue.' *J. Immunol.*, **90**, 595.

KAPLAN, M. H. and SUCHY, M. L. (1964). 'Immunologic relation of streptococcal and tissue antigens. II. Cross-reaction of antisera to mammalian heart tissue with a cell wall constituent of certain strains of group A streptococci.' *J. exp. Med.*, **119**, 643.

KAPLAN, M. H. (1965). 'Autoantibodies to heart and rheumatic fever: the induction of autoimmunity to heart by streptococcal antigen cross-reactive with heart.' *Ann. N.Y. Acad. Sci.*, **124**, 904.

KIDD, J. G. and FRIEDEWALD, W. F. (1942). 'A natural antibody that reacts in vitro with a sedimentable constituent of normal tissue cells.' *J. exp. Med.*, **76**, 543; 557.

LYAMPERT, I. M., BELETSKAYA, L. W., BORODIYUK, N. A. and SMIRNOVA, M. N. (1962). 'Antibodies reactive with the human heart tissue in rabbit antistreptococcal sera.' (In Russian). *J. Microbiol. Epidemol. Immunobiol.*, **2**, 62.

LYAMPERT, I. M., GRIZLOVA, O. N., BELETSKAYA, L. W. and DANILOVA, T. A. (1964). 'Investigation of the autoimmune process in connection with the problem of pathogenesis of rheumatic fever.' (In Russian). *Problems of Rheumatic Fever*, **3**, 92.

LYAMPERT, I. M., DANILOVA, T. A. and BORODIYUK, N. A. (1966a). 'Investigation of autoantibodies to the heart tissue produced by immunization with group A streptococci.' *Proceedings of Symposia on Immunology. 'Club of Immunologists', Moscow.* (In Russian). p. 73.

LYAMPERT, I. M., DANILOVA, T. A., BORODIYUK, N. A. and BETLETSKAYA, L. W. (1966b). 'The mechanism of formation of antibodies to the heart tissue in immunization with group A streptococci.' *Folia biol. (Praha)*, **12**, 108.

LYAMPERT, I. M., VVEDENSKAYA, O. I. and DANILOVA, T. A. (1966c). 'Study on streptococcus group A antigens common with heart tissue elements.' *Immunology*, **11**, 313.

LYAMPERT, I. M., BORODIYUK, N. A., VVEDENSKAYA, O. I. and DANILOVA, T. A. (1966d). 'The role of microbial antigens common to the elements of human and animal heart tissue in the appearance of autoimmune reactions.' *IXth International Congress for Microbiology, Moscow*, p. 656.

MARKOWITZ, A. S. and LANGE, C. F. (1964). 'Streptococcal related glomerulonephritis. I. Isolation, immunochemistry and comparative chemistry of soluble fractions from type 12 nephritogenic streptococci and human glomeruli.' *J. Immunol.*, **92**, 565.

MAURER, P. H., GERULAT, B. F. and PINCHUCK, P. (1964). 'Antigenicity of polypeptides (poly alpha amino acids). XII. Immunological studies with a new group of synthetic copolymers.' *J. exp. Med.*, **119**, 1005.

MAZINA, N. M. and RASSOKHINA, I. I. (1966). 'Concerning antigenic similarity of human heart tissue and streptococci' (In Russian). *Problems of Rheumatic Fever*. **1**, 8.

MEKLER, L. B. (1965). 'Genetic information used during the synthesis of virus components and antivirus immunity.' *Nature (Lond.)*, **206**, 343.

PERLMANN, P., HAMMARSTROM, S., LAGERCRANTZ, R. and GUSTAFSSON, B. E. (1965). 'Antigen from colon of germ free rats and antibodies in human ulcerative colitis.' *Ann. N.Y. Acad. Sci.*, **124**, 377.

ROWLEY, D. and JENKIN, C. R. (1962). 'Antigenic cross-reaction between host and parasite as a possible cause of pathogenicity.' *Nature (Lond.)*, **193**, 151.

TOMASI, T. B. (1961). 'A precipitation reaction between human serum and a soluble tissue component.' *J. Immunol.*, **86**, 427.

WEIGLE, W. O. (1963). 'Autoimmunity and termination of acquired immunological tolerance.' *IIIrd International Symposium on Immunopathology*, (Ed. by P. Grabar and A. Miescher), p. 167. Schwabe, Basel.

WEIGLE, W. O. (1965). 'The antibody response in rabbits to previously tolerated antigens.' *Ann. N.Y. Acad. Sci.*, **124**, 133.

ZABRISKIE, J. B., FREIMER, E. H. and SEEGAL, B. (1964). 'An immunological relationship between streptococcal membranes and human heart tissue.' *Fed. Proc.*, **23**, 343.

ZABRISKIE, J. B. and FREIMER, E. H. (1966). 'An immunologic relationship between the group A streptococcus and mammalian muscle.' *J. exp. Med.*, **124**, 661.

ZILBER, L. A. and ABELEV, G. I. (1962). *Virology and Immunology of Cancer*. Medical State Publisher, Moscow. (In Russian).