that described after strenuous muscular exercise.[5–10] Several authors have found that the elevation of enzymes is proportional to the severity and duration of physical exertion.

Ciaccheri and Maddaluno [1] and Mach et al.[2] noted that the serum-aldolase rose highest in their fatal cases of tetanus and tended to be normal or only slightly raised in the milder cases. In both our patients the serum-aldolase levels were elevated, but the rise in creatine phosphokinase was more striking. The latter enzyme is probably a more sensitive index and may be of greater value in the diagnosis of milder cases.

Further studies are required to try and correlate creatine-phosphokinase levels with clinical severity and prognosis. Furthermore, the effect of curarisation on the serum-enzyme levels in tetanus would be of interest.

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Hypothesis

THE ÆTIOLOGY OF SYSTEMIC LUPUS ERYTHEMATOSUS *

IN 1942, Klemperer et al.[11] pointed out that systemic lupus erythematosus (S.L.E.) was a disease of membranes. Although they coined the name " collagen disease " for S.L.E. and certain other diseases involving connective tissues, their use of this term did not imply that the initial lesion involved the protein collagen. Their earlier paper [12] emphasises that the initial lesion involves ground substance rather than collagen. This ground substance is the matrix of the basement membranes of glomerulus, skin, blood-vessels, pleura, pericardium, and endocardium. The ætiology of S.L.E. must therefore explain this lesion of basement membranes.

Other important findings in S.L.E. are elevated γ_2-globulins and the presence of antibodies to nucleoproteins. High levels of γ_2-globulin may be found in chronic infections, especially granulomatous infections.[13] However, even staphylococcal infections can produce high levels. We recently had a patient with a large staphylococcal abscess with a γ-globulin of 3·7 g. per 100 ml. The level fell to normal six months after drainage.

PRODUCTION OF HIGH LEVELS OF GAMMA-GLOBULIN

Studies in Sweden over twenty years ago provided information on the types of antigens that produce high globulins. Bjørneboe [14] and Bjørneboe and Gormsen [15] immunised rabbits intensively with three types of antigens. The normal globulin in rabbits was 1·23 g. per 100 ml. with a range of 0·48 to 1·85 g. After intensive immunisation with polyvalent pneumococcal vaccine, the rabbits developed levels of 3·5 to 4·5 g. of globulin as early as ten days after the beginning of immunisation. A maximum level of 7·1 g. per 100 ml. was found. Other rabbits immunised with polyvalent salmonella vaccine developed similar levels with a maximum of 7·9 g. All these animals showed intensive plasma-cell proliferation in many tissues. Still other rabbits were intensively immunised for almost three months with a mixture of horse, ox, and sheep serum. In contrast to the other groups, the mean post-immunisation globulin value was only 2·0 g. per 100 ml., with a maximum of 3·0 g. Relatively few plasma-cells were found in the tissues. The maximum amounts of protein injected with the pool of horse, ox, and sheep serum were 446 to 936 mg. This is a great deal more than was present in the bacteria.

For the pneumococcal vaccine it can be calculated [16] that the maximum mass injected was some 50 mg., while for the salmonella vaccine the maximum can be calculated to be only 10 mg.[17] While these workers did not isolate specific globulins, there is adequate information that the major response to bacterial antigens is an increase in γ_2-globulin.[13]

It is well known that the important antigens of the pneumococci are the capsular polysaccharide and the cell-wall C carbohydrate.[18] In the salmonella the important antigens are the carbohydrate Vi antigen [19] and lipopolysaccharide O antigen.[20]

The finding that carbohydrate antigens of bacteria are the most effective producers of γ-globulin and plasma-cells is consistent with other information on these antigens:

Protein antigens produce rapid antibody responses that fall rapidly,[21] but polysaccharide antigens maintain good antibody titres for long periods.[22] Poliovirus vaccine is a nucleoprotein which behaves as a protein antigen,[23 24] clinically effective in very small doses. A single injection of 1·2 μg of poliovirus type II produced in children $1{\cdot}2 \times 10^{-2}$ g. of antibody in the child.[24] 30 μg. of type-I pneumococcal polysaccharide produced 1·3 g. of antibody in men.[22] The antigen-dose/antibody-response relationship is very flat for polysaccharide antigens.[23 24] Therefore 1·2 μg of polysaccharide will produce 0·73 g. of antibody per man. The ratio of antibody formed per unit mass of antigen is therefore 61 times as great for the polysaccharide as for the protein. If correction is made for the difference in body-weight between children (24 kg.) and adults (70 kg.), the polysaccharide is 105 times as potent as the protein. Therefore both peak antibody titres and maintenance of these high titres are much greater for polysaccharide antigens than for protein antigens. This great effectiveness of polysaccharide antigens may well be due to the fact that bacterial polysaccharides are degraded far more slowly than protein antigens.[16 25] Though opinions differ on whether small amounts of antibody can be produced in the absence of antigen, there is no doubt that formation of antibody in large amounts requires the presence of antigen.

The obvious conclusions are that polysaccharide-containing antigens of bacterial origin are by far the most effective stimulants of plasma-cell proliferation and of γ-globulin synthesis. In contrast, plasma proteins are poor stimulants of these components.

S.L.E. AS AN AUTOIMMUNE DISEASE

Current concepts about S.L.E. are often expressed in terms of the clonal theory of antibody formation.[26] This

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theory [27] postulates that groups of lymphoid cells arise by mutation, and in S.L.E. such clones of cells produce immunologically competent cells which, either directly or through the production of antibody, react with body constituents producing the observed damage. There are two models which simulate such a condition. The best is runt disease.[28] Neonatal mice injected with adult splenic cells from a homologous donor will become tolerant to skin homografts from the donor strain. However, the foreign spleen cells injected neonatally are not tolerant of the host and react causing death. Since runt disease has been presented as a model for autoimmune diseases,[29] we should expect that the γ-globulin would be considerably elevated. Oliner, Schwartz, and Dameshek [30] studied runt disease in mice. They showed that out of ten groups developing runt disease only one (of four animals out of 106) demonstrated a significant increase in γ-globulin even though the mortality was in the range of 85%. Yet these animals were supplying huge quantities of innumerable antigens to the lymphoid cells, and hypertrophy of spleen and lymph-nodes demonstrated immune activity.

Another related system is that of homotransplantation. Tissue from a foreign host is introduced into a normal recipient. In time the graft is rejected. Since the immune system of the host is normal, we should expect an increase in γ-globulins as is found in S.L.E., if the two are analogous. The actual findings do not substantiate this. Homografts of skin to mice, guineapigs, and rabbits produced no significant increase in γ-globulins.[31] Furthermore, kidney homotransplants in dogs produced no impressive increases in γ-globulin.[32]

We must therefore conclude that most experimental systems which have been considered as analogous to autoimmune disease in man, typified by S.L.E., do not show the elevation in γ-globulin so common in S.L.E. This does not suggest that runt disease and homograft rejection are not immunologically mediated; it merely supports the prevailing opinion that these reactions are mediated by lymphoid cells rather than circulating antibody.

THE L.E.-CELL FACTOR

As mentioned earlier, the formation of antibodies which react with D.N.A. and nucleoproteins is the usual finding in S.L.E.; it is the basis of the L.E.-cell test. These antibodies are typical γ_2-globulins and cross-react with D.N.A. of mammalian, fish, and bacterial origin.[26] Although it is often assumed that the actual antigen is D.N.A., there is no direct evidence for this; D.N.A. is more likely a haptene with D.N.A. protein serving as the antigen. By any standard, animal nucleoproteins (N.P.) are poor antigens. Repeated injections in complete adjuvant is the usual requirement for antibody production.[33 34] Even large amounts of homologous and heterologous sperm given in complete adjuvant appear to be non-antigenic in rabbits.[35] In contrast, bacterial nucleoprotein will produce antibodies reactive with D.N.A. when injected without adjuvant.[36]

A study by Miescher et al.[34] is particularly informative. They immunised rabbits and guineapigs with nucleoprotein from calf thymus, calf liver, or brucellæ. Complete adjuvant was used; the amounts of mammalian nucleoprotein injected were much greater than the brucellar nucleoprotein. As assayed by the sensitive passive cutaneous anaphylaxis (P.C.A.) test, calf-thymus antigen was shown to produce serum titres of 1/5 to 1/25 while the brucellar antigens produced titres of 1/125 to 1/250. More importantly, complement-fixation tests showed that antisera to thymus nucleoprotein gave low cross-reactions with brucellar nucleoprotein, whereas antisera to brucellar nucleoprotein gave as high titres to thymus nucleoprotein as the thymus antiserum gave with the homologous antigen (thymus). In addition, brucellar nucleoprotein produced antibody in all animals while mammalian nucleoprotein did not. This low antigenicity of any type of nucleoprotein precludes these antigens as being responsible for the large increase in γ-globulin found in S.L.E.

These findings support the expected conclusion that bacterial nucleoprotein is more antigenic in mammals than mammalian nucleoprotein. They further indicate that strong cross-reactions occur between mammalian and bacterial nucleoprotein.

RELATION TO RHEUMATIC FEVER AND GLOMERULONEPHRITIS

We must now consider two diseases whose relationship to streptococcal antigens is firmly established—rheumatic fever and acute glomerulonephritis.

The work of Kaplan and his colleagues [37 38] leaves no reasonable doubt that cell walls of certain group-A β-hæmolytic streptococci share antigenic determinants with the heart. Of particular interest is the finding by both Kaplan et al. and Hess et al.[39] that the chief reactive antigen is in the sarcolemma and not the muscle. Therefore rheumatic fever is a disease in which cell walls of streptococci share antigens with the membranes of heart muscle, and presumably of synovial membranes. The specific streptococcal antigen(s) has not been chemically characterised. Since prophylaxis with penicillin is of dramatic value in preventing recurrences of rheumatic fever,[40 41] it is unnecessary to involve autoimmunity; all recurrences could be due to reinfections with the ubiquitous streptococcus. The rise in antistreptolysin-O titre in active rheumatic fever would be quite analogous to the lupus-erythematosus factor. In rheumatic fever, antibody to a protein enzyme of streptococci is formed which is of no pathogenetic significance but is of diagnostic value.

Likewise the studies of Markowitz and Lange [42] leave no doubt of shared antigenic determinants in cell walls of type-12 group-A β-hæmolytic streptococci and the basement membrane of kidneys. They demonstrated that the streptococcal cell membrane and the kidney basement membrane antigens contained glycoproteins. In both, the carbohydrate moiety contained glucose, galactose, glucosamine, and galactosamine.

Since two diseases of membranes are caused by antibodies induced by bacterial antigens, probably polysaccharides, it is reasonable to suppose that the most diffuse disease of membranes, S.L.E., is also induced by bacterial polysaccharide antigens.

Mammalian membranes contain hyaluronic acid which is a combination of N-acetylglucosamine and glucuronic acid.[43] N-acetylglucosamine is also found in the cell walls of streptococci groups A–F and in many other bacterial cell walls including the lactobacilli.[44] Furthermore, McCarty [45] has shown that the antigenic specificity

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of streptococcal polysaccharides largely depends on terminal N-acetylglucosamine radicals.

THE CAUSATIVE AGENT IN S.L.E.

These considerations make it seem highly probable that bacterial polysaccharide antigens are responsible for S.L.E. Similarity between the sequence of N-acetyl-glucosamine end-groups in human membranes and bacterial cell walls is probably the basis for cross-reacting antibody. That antibody is deposited on host membranes is demonstrated by the finding of γ-globulin attached to the kidney basement membrane in S.L.E.[46] The L.E. factor is a response to bacterial nucleoprotein and probably plays a negligible part in pathogenesis.

Gamma-globulin levels are usually much higher in untreated active S.L.E. than in active rheumatic fever or glomerulonephritis. It must follow that larger quantities of antigen(s) are present in S.L.E. In the other two diseases, we know that the source of antigen is usually a pharyngitis caused by streptococci. Characteristically the infection is fairly mild and therefore the number of bacteria involved must be relatively few. S.L.E. has never been clearly associated with streptococcal or other infections. The magnitude of the antibody (γ-globulin) response would require a great many bacteria; such numbers infecting tissue would produce severe clinical symptoms which could not have been missed through the years. There remains only one source for these quantities of antigens. They must be present among the normal flora of the oropharynx, colon, or vagina. The broad γ-globulin peak found in S.L.E. suggests multiple antigens. In general, gram-positive bacteria are richer sources of carbohydrate antigens than are gram-negative bacteria.

S.L.E. is almost entirely a disease of women of child-bearing age. One possibility for this selection could be that women during this period harbour a peculiar flora. This is indeed the case; large numbers of gram-positive lactobacilli are present in the vagina only during the thirty-odd years when regular menstrual activity is present.[47] If the role of lactobacilli is substantiated, these bacteria can be essentially eliminated from the vagina by withdrawal of œstrogens.

Genetic factors are clearly important in S.L.E.[48] This in no way negates the thesis since genetic factors are of great importance in rheumatic fever [49] as well as in such a clear-cut infectious disease as tuberculosis.[50]

SUMMARY

Critical analysis of systemic lupus erythematosus indicates that this disease, like rheumatic fever and glomerulonephritis, is caused by cross-reactions between antibodies to bacterial polysaccharides and similar chemical groupings of the polysaccharides in body membranes. Group-A β-hæmolytic streptococci do not appear to be involved. The source of the antigen(s) is probably the gram-positive normal flora. Lactobacilli are suggested as a prime suspect.

ACKNOWLEDGMENTS

This week marks the 65th birthday of Sir Macfarlane Burnet. As a former student of his, I owe him a great deal and dedicate this work to him. My conclusions are at variance with his on this subject. It is therefore fitting that I close with a quotation which he used to introduce his recent book on autoimmune diseases [26]:

"Where there is much desire to learn there of necessity will be much arguing, much writing and many opinions: for opinion in good men is but knowledge in the making"—JOHN MILTON, *Areopagitica*, 1644.

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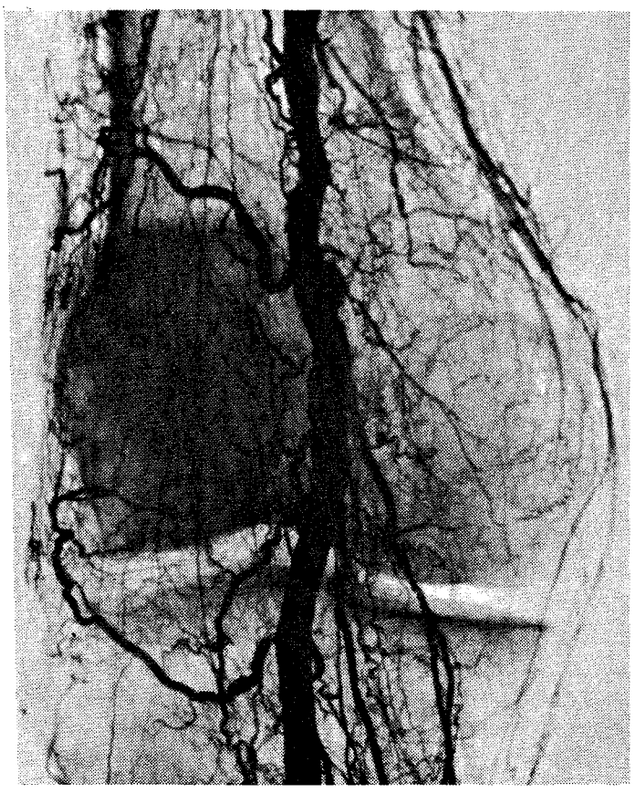
New Inventions

A NEW X-RAY CONTRAST MEDIUM FOR DISPLAYING CADAVER VESSELS

INTEREST has lately been renewed in the postmortem study of the arterial system, especially with regard to the blood-supply of tumours and the vessel changes in "failed" organ transplants. A very simple X-ray contrast medium has been devised which appears to display details of the smaller vessels better than do other preparations in common use.

The medium consists of commercial powdered red lead 1 part, and 'Lux' detergent (Lever Bros.) undiluted, $2^1/_2$ parts, measured by volume. Mixed immediately before use, it may be injected either with a syringe or with a pressure bottle, though the reservoir of the pressure-bottle will need to be shaken from time to time.



Anastomoses around the knee shown by injection of the femoral artery.

The X-ray was taken 2 minutes after injection. The patient, a man of 84, had been dead for 24 hours.

The liquid detergent holds the heavy powder in suspension, while its very low surface-tension and miscibility with aqueous fluids allows rapid filling of the finer vessels. The combination of good penetrating powder with a high degree of radio-opacity enables details of the smaller arteries to be shown with exceptional clarity.

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