RHEUMATIC FEVER: BIOCHEMICAL AND HISTOPATHOLOGICAL ASPECTS

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In rheumatic fever, lesions occur in the connective tissue—of the heart, of the synovial and serous membranes, and of the subcutaneous tissue. While these lesions are sufficiently characteristic to allow confident recognition on histological grounds alone, purely morphological studies have so far revealed little of the essential nature of the lesion, or its possible origins. Aetiologically, the implication of haemolytic streptococcal respiratory infections rests on sound inference from numerous bacteriological studies. Further, lesions more or less closely resembling those of the human disease have been produced in laboratory animals by techniques involving immunization or infection with streptococci, and also by non-specific sensitization to various foreign proteins. But whether or not immunologically determined, the primary lesion of the connective tissue in rheumatic fever, as distinct from the inflammatory reaction to it, remains undefined.

The recent tendency to include rheumatic fever in the group of 'collagen diseases,' along with rheumatoid arthritis, peri-arteritis nodosa, disseminated lupus erythematosus and a number of other conditions affecting connective tissue, is based on relatively slender evidence that collagen is, in fact, primarily affected. Apart from collagen fibres, connective tissue contains other extracellular elements—the elastic fibres and the inter-fibrillar ground substance. Although the latter has the appearance of an amorphous matrix, the work of Day (1950, 1952) suggests that it, no less than the fibrillar elements, has organized structural form. It is this framework of structurally organized connective tissue that is modified by the rheumatic lesion. Inflammation, as Ungar has recently re-emphasised (Ungar, 1952), is a *response* to a direct physical or chemical stimulation of the tissues. It is, perhaps, a reasonable working hypothesis, that this stimulus arises in rheumatic fever from changes occurring in the structural elements of the connective tissue. We know little of the nature of the changes occurring in fibrils or ground substance because we know

FIG. 1.—Fibrinoid necrosis in acute rheumatic fever. H. & E. subcutaneous tissue from elbow region of a girl aged six. Palpable nodules were not present. (By courtesy of Dr. L. E. Glynn)

little of their biochemistry; it is aptly said that in rheumatic fever the key host problem is to determine the biochemical lesion (Coburn, 1950). Some aspects of recent work relating to this subject have been discussed below.

Fibrinoid Necrosis: Histochemical Analysis

A variety of lesions, including some of those found in the group of collagen diseases, show areas of red fibrillar and amorphous material in sections stained with haematoxylin and eosin. In rheumatic fever the subcutaneous nodules, for

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TABLE I
HISTOCHEMICAL CHARACTERIZATION OF FIBRINOID

	Fibrin	Fibrinoid	Collagen
Structure	Granular	Fibrous refractile	Fibrous
Eosin	Red ++	Red ++	Red +
Phospho-tungstic Acid haematoxylin	Blue	Bluish	Grey
Metachromatic dyes	+	+	0
Periodic Acid Schiff	+	+	0
Tryptic digest	Attacked	Resistant	Resistant
Pepsin	Disintegrated	Slightly resistant	-
Pectinase	Loss of argyrophilia	Loss of P.A.Schiff	-
Collagenase (Welch)	Digested	Digested	Digested
Hyaluronidase (testic)	0	0	-

Modified from Glynn and Loewi (1952).

example, characteristically show a central core of this fibrinoid material; it has been observed, moreover, in the subcutaneous tissue at an early stage in the rheumatic attack before the appearance of palpable nodules (Fig. 1). The fibrinoid area is surrounded by a cellular inflammatory reaction, and there is good reason to believe that the site of fibrinoid change is identical with that of the primary lesion. It is important, therefore, to determine which components of the connective tissue are affected by this process and what change they are undergoing.

The designation 'fibrinoid' emphasises the similarity in staining properties of this material and fibrin, although Neumann (1896), who first

described it, believed fibrinoid to be a separate entity. Several workers have held that fibrinoid is simply connective tissue—that is, collagen fibres and ground substance—infiltrated with fibrin. The use of special stains such as Hotchkiss' periodic-acid-Schiff reagent method (P.A.S.) and Mallory's phosphotungstic acid haematoxylin have been of little help in resolving this problem, since fibrin alone gives colour reactions almost identical with those of fibrinoid. Altshuler and Angevine (1949) drew attention to the metachromatic staining given by fibrinoid with toluidine blue, and attributed this to the presence of precipitated acid polysaccharide, but the fact that metachromasia is readily observed in normal

tissues throughout the body obscures the particular significance of this reaction in fibrinoid. Indeed, the use of histological stains alone as reagents for identifying chemical entities is hazardous. Schlossman (1942) suggested that the use of enzymatic digestion, coupled with staining reactions, might advance this problem and, from his own work on sclerotic arteries, concluded that fibrinoid was not identical with fibrin. More recently Glynn and Loewi (1952) have confirmed and extended this work (Table 1). They find that the fibrinoid of rheumatic fever, unlike fibrin, is resistant to trypsin, which leaves the reactions with P.A.S. and Mallory's stain unaltered. They find, too, that fibrinoid behaves like collagen when treated with *Cl. Welchii* collagenase, although it is less susceptible than normal collagen to pepsin. Silver impregnation reveals the fibrillar nature of fibrinoid, and the fibrils themselves are strongly argyrophilic. Pectinase (a wide-spectrum polysaccharidase), or extraction with polysaccharide solvents, both have the effect of destroying the argyrophilia and reversing the positive P.A.S. reaction. It seems reasonable to conclude, therefore, that the characteristics of fibrinoid are independent of the presence of fibrin and also that, while collagen is an important constituent of fibrinoid, it may be somewhat altered and is infiltrated with a polysaccharide-rich material, probably not exclusively fibrin.

The Chemistry of Connective Tissue

The results of histopathological studies suggest changes in the rheumatic lesion affecting both protein and polysaccharide structures. The lesions of rheumatic fever affect predominantly sites related to movement—heart valves, joints, bursae and subcutaneous tissue. It is possible that the ground substance components which specifically subserve the purposes of movement—for example, hyaluronic acid in joint fluids—are especially susceptible to the chemical injury which, we have postulated, underlies the rheumatic lesion. Certainly in acute rheumatic fever the viscosity of the synovial fluid in affected joints is somewhat reduced. Although Guerra's claim (1946) that the skin of rheumatic fever patients permitted enhanced spreading with hyaluronidase has not been confirmed (Harris and Friedmann, 1949; Jaworski, *et al.*, 1950), there is evidence that in both acute rheumatism and rheumatoid arthritis the reconstitution of the skin substrate after enzyme injection is delayed (Bywaters, *et al.*, 1951).

The investigation of the polysaccharides present in connective tissue and their chemical characterization is still at an early stage. Some of the better-known animal polysaccharides have been

isolated from various human tissues; hyaluronic acid, for example, from vitreous humour and umbilical cord, and chondroitin sulphate from cartilage, heart valves and tendon. Meyer (1950) has described three types of chondroitin sulphate occurring in normal tissues which differ in their source, in their physical properties and in their type of linkage to protein. Little information is available about the chemical nature of tissue polysaccharides and proteins, but quantitative analysis and the use of chromatographic and ionophoretic methods have recently been used jointly to elucidate this important aspect. Collagen and elastin are the only animal proteins rich in hydroxyproline, and they may be estimated by determination of this amino-acid (Neumann and Logan, 1950). Tryptophan, on the other hand, which is absent in these two, is present in most other proteins and affords a measure of them. Collagen and elastin may be separated by autoclaving tissue with water, a process which yields a soluble, collagen-containing fraction, and an insoluble residue which includes elastin.

Consden, *et al.* (1953) have applied this technique to subcutaneous nodules obtained from rheumatic fever cases, and compared the extracts and residues with those from normal human subcutaneous tissue (Table 2). Untreated tissue extracts and residues were hydrolysed and examined chromatographically for amino-acids. The carbohydrates were examined by paper ionophoresis and subsequent paper chromatography carried out at right-angles to the direction of ionophoresis. These results throw new light on the chemical nature of the fibrinoid material of the rheumatic lesion. Neither chromatography, nor tryptophan and hydroxyproline analysis, showed much difference between rheumatic and non-rheumatic extracts. While chromatograms of non-rheumatic residues were characteristic of elastin, however, those from residues of rheumatic nodules showed the presence of an additional protein. Thus the hydroxyproline values, if attributed to elastin, were equivalent to 50 to 70 per cent. of the nodule residue. The tryptophan content, if due to fibrin, corresponded to about 30 per cent. fibrin in the nodule. A mixture of fibrin and elastin in these proportions, however, would give a tyrosine figure much lower than that actually found (Table 2). Further, while extraction by autoclaving dissolved about 50 per cent. of a fibrin clot, yet the tryptophan value in nodule extracts was much lower than would correspond with such a fibrin content. It seems likely, therefore, that fibrin, if present in the fibrinoid material of rheumatic lesions, is present in no great amount.

Consden, *et al.*, further found that, while tyrosine and sugar contents of all residues,

TABLE II
ANALYSIS OF SUBCUTANEOUS ELBOW TISSUE.

Modified from Consden, Glynn & Stanier, 1953.	Rheumatic Fever		Control		For comparison		
	Extract 70%	Residue 30%	Extract 91%	Residue 9%			
Chromato- gram type.	Coll- agen	Fibrin- Elastin	Coll- agen	Elastin	Coll- agen [†]	Elas- tin [†]	Fib- rin
OH-proline	7.6	0.7	7.7	0.9	8.0	1.0	0
Tyrosine	1.6	3.4	0.9	3.5	0.5	0.6	2.7
Tryptophane (N) as % total N)	< 0.1	0.8	< 0.1	-	0	0	2.7
Reducing sugar *	3.1	3.6	2.2	2.3	0.4	-	4.5
Hexosamine (Gluco- samine) (g/100g. protein)	0.9	0.9	0.5	-	0.2	-	1.1
Uronic acid.	0	0	0	0			

* Mainly galactose, some glucose & mannose.

[†] (Collagen and elastin figures from Bowes and Kenten (1949)
and Neumann and Logan (1950).)

rheumatic or non-rheumatic, were high, the amount of residue from rheumatic material was much greater (Table 2). Tyrosine, like tryptophan, measures non-collagenous protein. It seems, therefore, that both non-collagenous protein and polysaccharide occur locally in greater amounts in rheumatic nodules than in normal tissue. The nature of this polysaccharide is interesting. The sugars observed in normal tissues were predominantly galactose and glucosamine. Hyalobiuronic acid and galactosamine were not found in appreciable quantities, although if hyaluronic acid and chondroitin sulphate are present in subcutaneous connective tissue they might be expected to yield these substances. The results obtained suggest that in the type of connective tissue examined a galactose-rich polysaccharide is present and perhaps predominant. Identification of this entity is awaited with interest;

in the meanwhile, interpretation of chemical structure in terms of tissue-staining reactions proceeds with renewed caution.

The Structure of Collagen

In the 'collagen diseases' focal swelling and degeneration of collagen fibres has been frequently described. Gross (1950) has pointed out, however, that histological alterations in collagen fibres cannot be ascribed to changes in the collagen itself unless the abnormality can be localized in its component fibrils. Electron microscopy of the collagen fibrils in this group of diseases has, however, given rather disappointing results. Thus Gale (1951), Gross (1952) and Ball, *et al.* (1951) were unable to demonstrate abnormal fibrils in the nodules or lesions of rheumatoid arthritis, rheumatic fever or disseminated lupus erythematosus. The appearances of normal collagen in

the electron microscope are striking; fibrils show a regular periodic crossbanding at $640\text{\AA}$ intervals, with a characteristic complex of intraperiodic sub-banding. It seems reasonable to expect that this appearance must be modified if disease is located in the fibril itself. So far, however, convincing changes in the fibrils have been demonstrated only in senile skin and in some rare skin conditions (Tunbridge, *et al.*, 1952). Wolpers (1950) encountered non-banded fibrils in rheumatic nodules, but only in the necrotic areas, and considered that the change followed, rather than preceded, the development of the fibrinoid lesion. Recently, however, Rich, *et al.* (1953) have described markedly abnormal collagen fibrils occurring in experimentally produced local anaphylactic skin lesions of the Arthus type, and have pointed out certain technical difficulties in demonstrating their presence. Electron microscopy may have much more to reveal about the fine structure of collagen in health and disease.

The biochemical approach is increasingly relevant to the problems of hypersensitivity and immunology and their relationship to the initiation of the tissue changes which precede the development of rheumatic lesions. Modern methods of microscopy and tissue analysis have contributed much to the understanding of connective tissue

structure and function. It is not unreasonable to hope that the elucidation of rheumatic disease lies within the same field.

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MEDICAL TREATMENT OF RHEUMATIC HEART DISEASE

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Widespread interest in the recent important advances in the surgical treatment of mitral stenosis has temporarily transferred emphasis from the medical aspects of the treatment of rheumatic heart disease. It must not be forgotten, however, that no matter how dramatic the improvement which follows operation, the patient still has rheumatic heart disease with myocardial and perhaps other valvular lesions, is still liable to the risks and dangers of that condition and has, indeed, still got mitral stenosis, though often of significantly less severity. The principles of management are not changed, even if the severity of the disease is mitigated.

Asymptomatic Rheumatic Heart Disease

A large proportion of patients with rheumatic heart disease are recognized in the asymptomatic stage; this includes most of those who have had a typical attack of rheumatic fever (about half the cases) and many others whose disease is discovered by mass radiography, by routine examination at school, on examination by National Service Medical Boards, at insurance examinations and during routine examinations during pregnancy.

Amongst such cases children left with a systolic murmur after rheumatic fever form an important group. Too often the physician advises restriction of activity in a misguided attempt to safeguard the

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