STRUCTURE OF ELASTIN

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1. Introduction

Unlike other components of the connective tissue, relatively few structural studies have been reported on elastin. The earliest to investigate the X-ray diffraction pattern of elastin was Kolpak (1935) who found that the unstretched ligament yielded diffuse rings, but on stretching these gave a typical collagen pattern with meridional arcs corresponding to a spacing of 3 A and equatorial spots corresponding to spacings of 11·5 A, 5·9 A and 4·6 A. Astbury (1938, 1940) attributed these results to the presence of small amounts of collagen as impurity in the specimens. According to him, the unpurified fibres of *ligamentum nuchæ* give only an amorphous pattern, but this may be superimposed with the pattern of imperfectly oriented collagen fibres. On stretching, the collagen pattern becomes well oriented. On the other hand, he found that the collagen pattern disappeared if the fibres were treated with hot water over a steam-bath for about a week. On stretching, the amorphous pattern of elastin also showed some signs of orientation parallel to the direction of stretching. Astbury suggested that elastin might be a member of the collagen group whose thermal transformation temperature is below room temperature, but no conclusive evidence was available at that time to decide the question. Bear (1944) recorded small angle patterns of collagen faintly with beef ligament and ascribed these to the presence of very small amounts of collagen as impurity. He suggested that elastin must be excluded from the collagen family (Bear, 1952). Electron microscopic studies (Wolpers, 1944; Gross 1949, 1951; Franchi and de Robertis, 1951) also did not reveal the 640 A spacing typical of collagen or any other fine structure in elastin. The present situation regarding the structure of elastin, as revealed by X-ray and other physical methods, may be summarised as follows (Kendrew, 1953): "Indeed the two main groups of the Astbury classification appear to comprehend virtually all known structure proteins whose X-ray pattern has been examined, with the exception of elastin (a component of some types of elastic tissue) and actin in muscle."

Among the other chemical and other studies on elastin, the following may be mentioned in particular. Lloyd and Garrod (1948) compared the thermoelastic behaviour of elastin and collagen and considered that the

high proportion of non-polar side chains present in elastin would account for the rubber-like properties. Hall *et al.* (1952) suggested that the elastin system at least in parts is made up of linear aggregates of a corpuscular protein unit of low molecular weight called proelastin, analogous to procollagen. Banga and Schuber (1953) have suggested that elastin is a glycoprotein, the fibrous character of which is determined by a carbohydrate group built into the molecule. Later Banga (1953) showed that the reducing substances split off during elastolysis could not be ascribed to carbohydrate. Partridge *et al.* (1955) have suggested that the substance of elementary elastin fibres is a chemically homogeneous protein, although it may exhibit heterogeneity at macromolecular level. The physical properties of the elastic tissue may be a result of the macromolecular structure of elastin comprising the elementary fibres and the way in which the fibres are cemented by collagenous structure and mucoproteins.

During a recent series of studies in this laboratory on the effect of chemical reagents on collagen, it was observed that some of the treated specimens exhibited a very close similarity to elastin in their X-ray pattern as well as in the physical behaviour (unpublished). The change thus produced in collagen was found to be reversible, so that the treated specimen should be considered to be only a modified form of collagen. A careful investigation was therefore made of the relationship between collagen and elastin and the present paper is a report of these studies. It appears that elastin should definitely be classed with collagen and that its molecular structure is also basically built up on the triple chain structure of collagen (Ramachandran and Kartha, 1954, 1955, *a*, *b*; Ramachandran, 1956).

2. X-Ray Patterns of Elastin and Collagen

The X-ray diffraction pattern of elastin supports the suggestion that elastin is closely related to collagen in its structure. The diffraction pattern of elastin, recorded in a 3 cm. cylindrical camera is given in Fig. 1. The pattern in Fig. 1 may be compared with the diffraction patterns of natural collagen fibres (Fig. 2), thermally shrunk collagen (Fig. 3) and gelatin (Fig. 4). The fibres of elastin were obtained from *ligamentum nuchæ* of cattle. The fibres were treated with 20 per cent. sodium chloride solution, washed with distilled water and dehydrated with acetone. Further purification was effected by autoclaving with water. Collagen fibres were obtained from cattle achilles tendon from which soluble proteins were removed by extraction with sodium chloride solution. B.D.H. gelatin was used for obtaining Fig. 4. The thermally shrunk collagen specimen was prepared by heating

a collagen fibre well above the shrinkage temperature and then fixing it in boiling alcohol.

The prominent features of the diffraction pattern of elastin are two diffuse rings of spacings 4·4 A and 2·2 A. While the former was recorded by Astbury using a flat film, the latter has now been observed for the first time. The pattern recorded on a flat film is shown in Fig. 5 and it will be seen from this that there is in addition a central halo, with a faint maximum at about 9–10 A. This feature has also been commented upon by Astbury (1940).

A comparison of Figs. 1 to 4 shows that the 2·2 A ring is common to all, although the other details differ. In fact, there is a close correspondence between the patterns of elastin and thermally shrunk collagen. However, in the patterns of collagen fibre and gelatin, several other reflections occur. Thus, the pattern of collagen fibre exhibits a sharp equatorial reflection at 11 A to 12 A and meridional arcs at 2·86 A and 4 A, and two off-meridional spots corresponding to a spacing of about 7 A. All these reflections occur in the form of rings in the pattern of gelatin. These features are not fully visible in Figs. 2 and 4 since the central region has been overexposed to bring out the 2·2 A ring. Thus, gelatin at room temperature may be considered to be a disoriented (polycrystalline) form of collagen.

The pattern of thermally shrunk collagen differs significantly from those of both normal collagen and gelatin. While the occurrence of complete rings indicates the absence of orientation, as in gelatin, there is a clear indication that it is a more degraded form of collagen than gelatin, since the sharp rings in the pattern of the latter are all absent in the pattern of thermally shrunk collagen.

The sharp reflections corresponding to definite spacings occur in the pattern of collagen fibres because of the high degree of order in its structure. This order is of two types:

(*a*) the order arising from the arrangement of the triple-chain protofibrils in a lattice—the lattice may be a simple hexagonal lattice, or a cylindrical lattice (Ramachandran and Sasisekharan, 1956) but the exact nature is not important for our discussion here.

(*b*) the order arising from the helical arrangement of residues in an individual triple-chain protofibril.

The former leads to the 11A to 12 A equatorial spacing, while the latter is responsible for the 2·86 A meridional arc and also the other spacings.

The 2·86 A corresponds to the tenth order of the repeat distance of 28·6 A spacing along the fibre axis.

It is obvious that the two types of order must be interrelated. An examination of the way in which the triple-chain structure is built up from the amino-acid residues and is stabilised reveals that the exact value of 28·6 A is not demanded by stereochemical or any other considerations connected with a single triple chain. A fairly wide variation, certainly from about 28 A to 31 A, is permissible. Thus, the occurrence of a sharp spacing of 2·86 A must be attributed to the cross-links between *different* protofibrils, which would lead to the repeat spacing being the same in all the protofibrils, and thus producing a high degree of regularity. If for any reason these cross-links are ruptured, then one should observe two effects—(*a*) the equatorial reflection would no longer be sharp, because there is no reason why the protofibrils should be maintained at a constant distance apart, (*b*) the individual protofibrils may also have different values for the repeat spacing along the fibre axis, so that the 2·86 A arc would be rendered diffuse and may even disappear. This is precisely what happens as a result of thermal contraction. The sharp equatorial spots in collagen fibre, which correspond to the 11 A to 12 A ring in cold gelatin, disappear on thermal contraction, and simultaneously the 2·86 A arc and the other sharp reflections or rings also disappear.

The fact that the broad rings at 4·4 A and 2·2 A, which are observed with gelatin, are present in the diffraction pattern of the contracted collagen suggests that the essential triple helical structure is maintained, except that the lattice structure and regularity are lost in the latter. This suggestion is further supported by the fact that thermally contracted collagen may be readily brought to the normal state by stretching it at room temperature.

As already mentioned, the diffraction pattern of elastin is closely similar to that of thermally contracted collagen, and so it is reasonable to suppose that the structure of elastin is also based on the triple-chain collagen structure, in which however the cross-linkages are not present. This happens because elastin contains very few residues with polar side-groups and in particular contains negligible amount of hydroxyproline (Partridge *et al.*, 1955), which is the main cross-linking agent in collagen.

3. Theoretical Considerations

The above observations strongly suggest that the structure of elastin and collagen are closely analogous at the molecular level, and that they are both built up on the triple-helical arrangement proposed from this laboratory (Ramachandran and Kartha, 1955; Ramachandran, 1956). This is

theoretically also very reasonable, for the amino-acid composition of elastin is similar to collagen in two important respects, although there are very few residues with polar side-chains in elastin. It contains slightly more than one-third of glycine and further the proline content is nearly the same as in collagen (Tristram, 1953). The triple-chain structure has the essential property that one-third of the α-carbon atoms cannot have any side-groups attached to them so that the glycine residues must form at least a third of the total number. This is characteristic both of collagen and elastin. So also the five-membered ring of the proline residue can be readily accommodated in the structure. It is interesting that both polyglycine and polyproline are found to take up a configuration similar to the collagen chain (Rich and Crick, 1955; Cowan and McGavin, 1955). Thus a close similarity is to be expected between the structures of collagen and elastin, although so far there is no evidence that every third residue in elastin is glycine or regarding the relative disposition of glycine and proline residues. However, it is possible that the number of residues per turn (which is very nearly $3\frac{1}{3}$ per turn in the minor helix) may not be exactly the same in elastin.

4. Relationship between Elastin and Chemically Treated Collagen

Recently the authors have made a study of the effect of various inorganic salt solutions like those of nickel nitrate and calcium chloride on collagen. Collagen contracts by treatment with these reagents even at room temperature. The progressive transformation of the structure brought about by these reagents has been followed by recording the X-ray patterns after treatment for various intervals of time (unpublished). In the case of both reagents, it is observed that there occurs first a disorientation of the structure and then both the 2·86 A arc and 11 A to 12 A equatorial spots simultaneously disappear. Just after this stage, the pattern of the treated fibres shows a remarkable similarity to that of elastin. Patterns of the calcium chloride treated collagen and of normal elastin recorded on a flat film at 3 cm. are given in Figs. 5 and 6. It will be noticed that the patterns are closely similar and that one could be easily mistaken for the other. The occurrence of a ring at about 9 A to 10 A at the outer edge of the central halo in the pattern of the treated collagen specimen (exactly as with elastin) is particularly noteworthy.

In the nickel nitrate treatment also, the same similarity is observable although there is a general background intensity probably due to the scattering and fluorescent X-rays from nickel in the pattern of this specimen. The ring at 9 A to 10 A has not been observed in the nickel nitrate treated

specimens if the concentration of the solution is large (100 per cent w/v), but the pattern given by them is closely similar to that of thermally shrunk collagen. If a dilute solution of nickel nitrate (30 per cent w/v) is used, and the collagen fibre is treated for a long time (several days), then the X-ray pattern of the fibre is very similar to that of the calcium chloride treated specimen, with a ring at about 9 A at the outer edge of the central halo.

All these specimens also give the 2·2 A diffuse ring if the patterns are recorded in a 3 cm. cylindrical camera. Usually the fibres begin to contract at the stage when the 2·86 A and 11 A to 12 A spots disappear, and they exhibit rubber-like elasticity at this stage.

Further treatment with concentrated nickel nitrate solution causes the disappearance of the halo sorrounding the central spot and the pattern shows only a diffuse ring at 4·4 A with very little intensity inside. This is identical with the pattern of drastically shrunk rat tail tendon recorded by Astbury (1944) and of gelatin sol (high temperature form of gelatin) recorded by Katz *et al.* (1931). Elastin, when treated with nickel nitrate solution for a long time, also gives a pattern identical with the above pattern (*see* Figs. 7 and 8). It may be mentioned that the collagen fibre gives the usual pattern, if it is washed in distilled water, whatever be the stage to which it had been modified by the chemical treatment.

These studies with chemically treated collagen confirm the ideas put forward in the previous sections. The fact that the treated collagen fibres can be readily restored to their normal condition by removing the salt means that the modification induced by the chemical treatment must be very mild. Since the X-ray pattern and physical behaviour of the specimen, treated under appropriate conditions, are very close to those of elastin, it is clear that elastin itself must be very similar to collagen. Further, it is observed that not only can collagen be modified further beyond this stage, when its X-ray pattern is similar to that of elastin, to that corresponding to hot gelatin, but elastin also exhibits such a change when treated with nickel nitrate. In fact, drastic thermal treatment also produces such a change in elastin, exactly as with drastically thermally shrunk collagen. If elastin is autoclaved and immediately the X-ray pattern is recorded, the pattern is identical with that of the drastically shrunk collagen, both showing only a fairly sharp ring at 4·5 A, with practically clear region within. This photograph is not reproduced, but is very similar to Fig. 7 (*a*) of Astbury's paper (1940) of rat tail tendon contracted to about 17 per cent. of its initial length.

Similarly, it has been observed that if elastin is kept wet and the X-ray pattern taken, the pattern is very similar to that of wet gelatin. Thus, not only is it possible to produce a material from collagen which is very similar to elastin, but the further changes produced in both are also very similar. All these clearly show that elastin in fact belongs to the collagen family of proteins.

5. OTHER OBSERVATIONS ON COLLAGEN AND ELASTIN

When the above studies were nearing completion, an interesting article by Burton *et al.* (1955) appeared, in which an apparent transformation of collagen fibres into a structureless elastin-like material was observed, when collagen was treated with some chemical reagents like borate buffer solutions. However, these authors have not observed the re-formation of the collagen, when the chemical is washed off.

The similarity of shrunk collagen and elastin has also been observed by Banga (1953) who found that collagen is attacked and solubilised by elastase if it has been shrunk thermally or by treatment with phosphotungstic acid, but is unattacked in the normal state.

In view of the evidences from different sources, it appears to be very likely that proteins with properties and composition intermediate between those of collagen and elastin could also occur in biological systems (*cf.* Burton *et al.*, 1955). It would be interesting to examine if this is so. The X-ray patterns of immature and developing collagenous tissue recorded by North *et al.* (1953) appear to support this idea. With increase of age of rats, the pattern of the tail tendon is found to change from the elastin-shrunk collagen type to the normal collagen type. So also the infra-red data of developing tendon given by Jackson *et al.* (1953) show that the NH stretching frequency (3310 $cm.^{-1}$) of the developing tendon is significantly lower than that of adult tendon (3330 $cm.^{-1}$) and agrees with the value observed for hot gelatin (Robinson, 1953). It would be interesting to examine if elastin itself shows the lower frequency since the present studies indicate the similarity of the structure of elastin to degraded and less developed forms of collagen. So far no infra-red data on elastin appear to have been reported.

Thus, elastin is probably a precursor of collagen in biological systems and collagen may be formed by the incorporation of polar residues and the resulting formation of cross-linkages. Burton *et al.* (1955) have on the other hand suggested that elastin is produced *in vivo* by degradation of collagen, although some of the co-authors (Hall *et al.*, 1955) themselves are of the view that this is probably less reasonable biologically than the reverse process.

6. Summary

The paper deals with the structure of elastin and its relationship to collagen. It is shown that, on the molecular level, elastin is analogous to collagen and is built up on a triple-chain helical arrangement of amino-acid residues. It is possible to obtain a material yielding an X-ray diffraction pattern very similar to elastin, by treating collagen with calcium chloride solution. The relationship between elastin and collagen in biological systems is also discussed.

7. Acknowledgement

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8. References

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DESCRIPTION OF PLATES

PLATE II

All the photographs have been taken with CuKα radiation in a crylindrical camera (R = 3 cm.)

FIG. 1. Elastin.

FIG. 2. Collagen fibre.

FIG. 3. Thermally shrunk collagen.

FIG. 4. Gelatin.

PLATE III

FIG. 5. X-ray diffraction pattern of calcium chloride treated collagen (Flat film, $d = 3$ cm.).

FIG. 6. X-ray pattern of elastin (Flat film, $d = 3$ cm.).

FIG. 7. X-ray pattern of nickel nitrate treated collagen (Flat film, $d = 5$ cm.).

FIG. 8. X-ray pattern of nickel nitrate treated elastin (Flat film, $d = 5$ cm.).

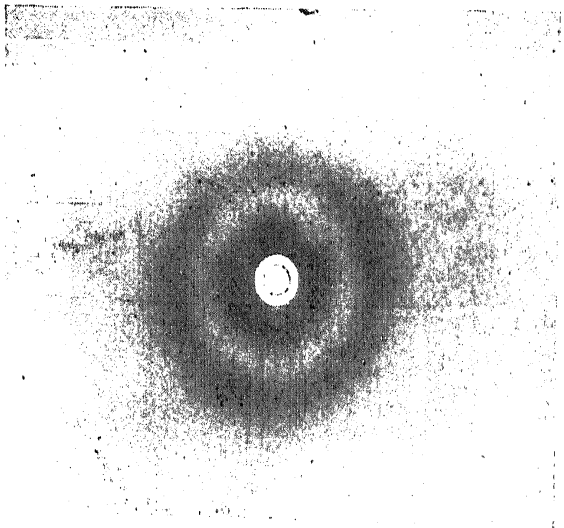

FIG. 1

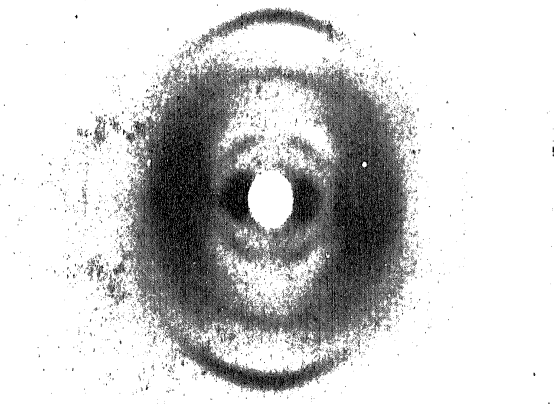

FIG. 2

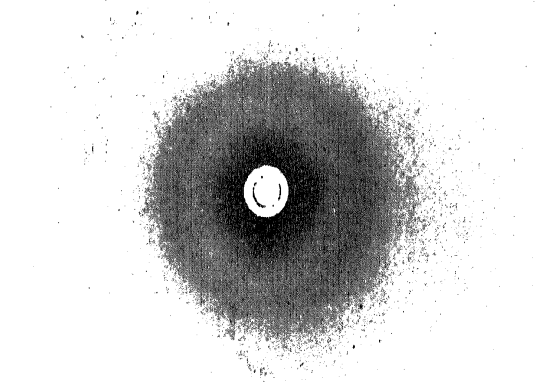

FIG. 3

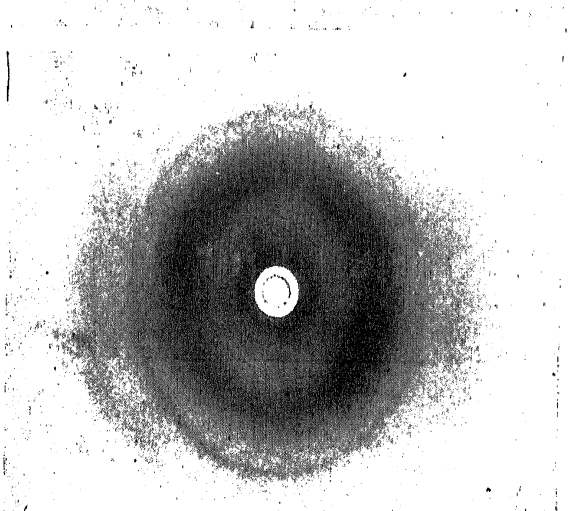

FIG. 4

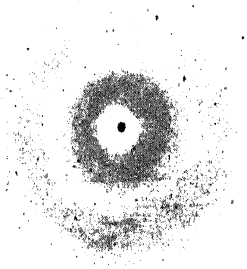

FIG. 5

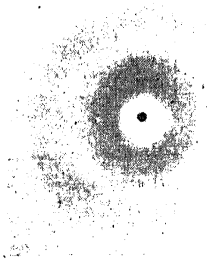

FIG. 6

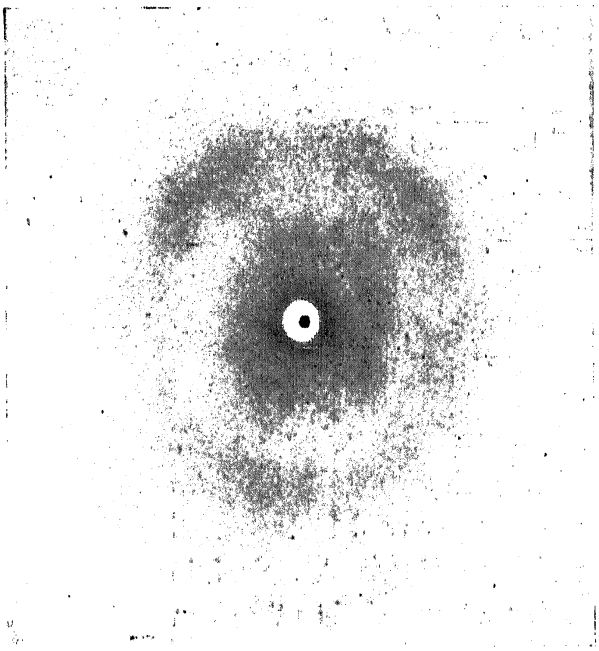

FIG. 7

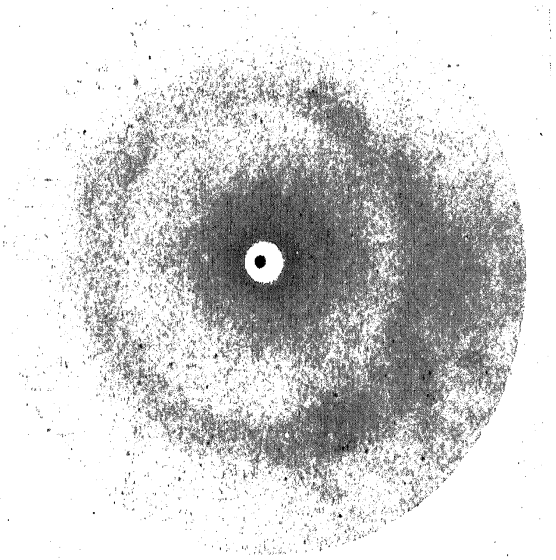

FIG. 8