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SUBMICROSCOPIC STRUCTURE OF COLLAGEN FIBRES: THEIR CONTRACTION AND RELAXATION

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In a previous communication [3] a brief account has been given of our observations relating to the thermal and chemical contraction and relaxation processes of collagen fibres. The thermal shrinkage of collagen fibre is an old known phenomenon but the chemical contraction-relaxation process was first observed by the present authors. PRYOR [9] described that tendons immersed in a solution of potassium mercuric iodide (K_2HgI_2) contract and display great tension during contraction, but he failed to observe relaxation to follow contraction. Adapting the experiments made with collagen fibres to muscle fibres and actomyosin threads, LAKI and BOWEN [6] showed that in potassium mercuric iodide contraction of the fibres is not followed by elongation, the latter supervening only in solutions of potassium iodide (KI). In our experience the contraction ensuing in KI solution and the relaxation that follows it, i. e., the process of chemical contraction-relaxation, is a phenomenon which presents a suitable means of studying the complex nature of the collagen fibres.

We have shown [4] that the crystallizable procollagen described by OREKHOVITCH et al. [7] is but one of the components of collagen fibres and that both this and the insoluble collagen designated by us as metacollagen are required to build up the collagen fibre capable of performing the combined function of contraction-relaxation. In the present paper the experimental data and results are discussed in detail which tend to prove that the collagen fibre consists of two principal protein components: procollagen and metacollagen. In addition, mucoproteins and nucleotides participate in small quantities in building up the collagen fibre, but it is not proposed to deal with them in the following.

Several methods have been applied in our experiments. The submicroscopic morphological changes related to contraction and relaxation were observed under the polarization microscope. Double refraction of native, contracted, and relaxed fibres was examined in the presence of different imbibition fluids. In applying this method, a procedure elaborated by one of us [10] was followed. The morphological changes obtained in thermal and chemical contraction-relaxation were brought into correlation with protein dissolution.

Also an enzymatic method was used to demonstrate the two kinds of protein components that make up the collagen fibre.

Methods

Collagen fibres obtained from tendons removed from the tail of 4 to 6 months old rats were used. (Fibres from animals more advanced in age yield different results ; we intend to discuss this point in a future report.) The animals were killed with gas ; from the skinned tail the tendons were removed. The individual collagen fibres were drown out from them in their entire length and were freed from adhering fragments of muscle and ground substance, using a dissecting needle. The individual fibres were washed with water then dried on filter paper. Fibres dried in an exsiccator were used in the chemical and enzymological experiments.

Fibres 60 mm long, 0,07 to 0,09 mm in diameter, weighing 0,5 to 0,8 mg, and loaded with 80 to 100 mg of lead shot were used for measuring thermal and chemical contraction-relaxation. These fibres displayed a uniform structure under both the normal and the polarizing microscope and appeared homogeneous with van Gieson's stain. Contraction-relaxation was carried out in part in a water bath at 67 °C, where the fibres contracted in 8 to 14 sec. and relaxed to their original length in 60 to 100 sec. Chemical contraction-relaxation occurred in a 40 per cent KI solution at 20 °C in which contraction started in 60 to 120 sec-s, and attained its peak between the 4th and 5th minute, while relaxation set in in 25 to 30 min. and was complete in 2 to 3 hours.

As regards the protein dissolution taking place during contraction-relaxation, on the analytical balance 20 mg of dry collagen fibres were measured into each of several tubes of known weight, 3 cm in length, and 0,5 to 0,6 cm in diameter. The total N contents of the fibres ranged between 16,6 and 16,8 per cent. After adding 2 ml of distilled water the tubes were immersed in a water bath of 67 °C observed. In dependence on the morphological changes the thermal action was interrupted at varying intervals by a rapid cooling of the tubes with ice water. Following centrifugation the fluid was decanted, the insoluble fibres were washed with alcohol and then dried at 110 °C. Thereafter we determined by the micro-Kjeldahl method the protein content of the decanted fluid ; the result was found to correspond to the difference between the initial weight of the collagen-containing tubes and their weight after drying at 110 °C.

The procedure followed in connection with the chemical contraction-relaxation process was to add 2 ml of a 40 per cent solution of KI to the 20 mg of collagen fibres in the tubes and, keeping the solution on them at a temperature of 20 °C, the changes occurring at different periods of time were observed. In contraction the markedly swollen fibres invariably displayed two distinct parts, each in a phase different from the other. The outer part was homogeneous, the inner, spiral. The appearance of the latter formed the basis for assaying contraction. During the transition from contraction to relaxation, the spiral part tapered gradually until it ultimately disappeared. This is when relaxation sets in whereafter the fibres appear once more perfectly homogeneous. It was possible to study these morphological changes under the hand lens, and interruption of the incubation in KI solution was regulated on dependence of these observations. Following incubation in potassium iodide the fibres were washed with water, then with alcohol, then dried at 110 °C. The difference in the weight of the untreated and the remainder of the treated fibres gave the amount of protein dissolved.

In our enzymological experiments, to collagen fibres treated as above with heat for different periods of time, or by incubation in a 40 per cent solution of KI an amount of 0,5 mg of elastase was added and with a n/10 Na_2CO_3—HCl buffer of pH 10 the tubes were made up to 2,5 ml. Incubation for 15 minutes at 37 °C followed, whereafter the insoluble fibres were centrifuged, washed with alcohol, dried at 110 °C, then weighed. The collagen dissolved by the enzyme was computed from the difference in weight of the untreated control tubes and the test tubes.

Results

Submicroscopic structure of collagen fibres under the polarization microscope

Collagen fibres are known to display intensive double refraction in polarized light. As seen in Fig. 1, under the polarization microscope, between crossed

nicols and a gypsum I lamella inserted in it, the collagen fibres give a highly positive double refraction.

In substances of a refractive index around 1,5 or 1,6 this double refraction is reducible to a minimum by imbibition, but cannot be eliminated completely. This proves that, in addition to formal double refraction, native collagen fibres also have an intrinsic double refraction.

Metacollagen shows less double refraction than native collagen does. From the point of polarization optics, difference must be made between metacollagen studied in the stretched and the shortened state. The double refraction of the elongated metacollagen is nearly of the same degree as the path difference measurable at the lowest point of the imbibition curve of native collagen. From this it seems permissible to infer that the component responsible for the higher double refraction in native fibres dissolves during contraction. This is the component which keeps metacollagen in a stretched state and in the absence of which the fibres shrivel up and become elastic and extensile, like elastic fibres. Formal double refraction of native collagen fibres is to a great extent due to this component.

There is another similarity to the elastic fibres which manifests itself in this state. In fat solvents and oils (xylene, toluene, olive oil, cedar oil) the double refraction of stretched metacollagen fibres is not lowered, as could be expected on grounds of the refractive indices of the said substances, as was the case with native fibres. It is only on the action of water and aqueous solutions that metacollagen loses its double refraction and that, simultaneously, the previously stretched fibre again shrinks. A perfectly similar phenomenon is observable in the elastic fibres.

It is quite probable that in both these cases it is upon the action of the elongation that the fibres, otherwise in isotropic phase, become anisotropic.

Therefore, the molecular double refraction observed during imbibition in native collagen and metacollagen should be regarded as the form in which metacollagen manifests itself in the anisotropic phase. As has been seen, this can be brought back to the isotropic phase at will, by interrupting elongation.

Placed in water of 67 °C or a 40 per cent KI solution at 20 °C, the collagen fibres contract in the time stated in the method. On discontinuing the heat effect, or lifting them from the solution the fibres stay contracted. As can be seen from Fig. 2, their polarization pattern differs essentially from that of native fibres. Separation into two phases each displaying a double refraction of different intensity can be observed. An apparently homogenous part shows little double refraction, while in an inner part of spiral structure the fibrillae lying at right angles to one another exhibit alternating positive and negative double refraction under the gypsum lamella. Quantitative measurement by means of a compensator shows the double refraction to decrease markedly

on the outer part of the fibre. In water, too, decrease of double refraction is encountered ; the outer part then is lost.

For the phenomenon described in the foregoing the following explanation is offered. On contraction the bond between procollagen and metacollagen, the two constituents of the fibre loosens and the fibrebuilding filaments that until then had been stretched, shrivel up. The mucoid having dissolved in the initial phase of contraction, the procollagen, too, begins to dissolve in an inward direction. In the part which is apparently isotropic the procollagen is completely dissolved while the fibrillary part represents the metacollagen. Fig. 3 presenting the picture between completely crossed nicols of a fibre imbibed with water clearly shows that only that part remains anisotropic in which dissolution of the procollagen is still incomplete. This means that the metacollagen which remains in the fibre after dissolution of the procollagen is no longer doubly refractile in the contracted state and so the particular spot remains dark under the polarization microscope.

With the thermal effect not interrupted, or the incubation in the 40 per cent KI solution not discontinued, contraction is followed by relaxation. In our view all the procollagen dissolves during relaxation and only the metacollagen is left over. The pattern obtained under the polarization microscope after the relaxation is in its entirety that of the metacollagen, but while on contraction the transformation into metacollagen is partial, in relaxation it is to be regarded as completed. Metacollagen fibres loaded with lead shot still show some double refraction after thermal or chemical treatment as a consequence of the tensile load. Fig. 4 presents the picture of an unloaded relaxed (metacollagen) fibre, the double refraction of which has completely ceased. This may be observed immediately after thermal or chemical relaxation. If the relaxed fibre is left to stand for a few hours, water takes the place of the procollagen which, substantially swelling the fibre, displaces it laterally and the originally positive double refraction turns into negative one.

Collagen fibres pretreated with a citrate buffer of pH 4 for dissolving the procollagen take up more water i. e., display a more marked neutral swelling, than native fibres. The thin delicate fibrils retain their parallel arrangement, provided they are handled carefully, but the fibre is unable to withstand even gentle stretching, compression, or tensional strain, unlike the native fibres, which are of great stability. If a metacollagen fibre prepared in this manner and imbibed with water is crushed with a glass rod, the delicate fibrils, as shown in Fig. 5, lose their arrangement parallel to the longitudinal axis, and at the same time their double refraction decreases.

The reason why following treatment with a citrate buffer of pH 4 the double refraction of the metacollagen prepared in this manner does not cease in water either, is that, although the procollagen has been dissolved, the mucoid still present prevents the fibres from passing into the isotropic phase.

These submicroscopic findings, and in addition, the contracting relaxing capacity of the collagen fibre seem to afford support to our view that the collagen fibre is built up of two kinds of structural protein, procollagen and metacollagen both of a particular functional significance.

Protein dissolution of collagen fibres during thermal and chemical contraction-relaxation

As has been pointed out in describing the "method", at a temperature above 67 °C collagen fibres contract rapidly, shortening to one third of their length. With the thermal action continued, after the lapse of a certain period of time, relaxation starts, the fibre re-extending to its original length. This process cannot be followed exactly unless lead shot is attached to the end of the fibres to keep them stretched and so enable one to make accurate readings of the changes in length with time. In a previous communication [4] it has been shown that the shortening of the native fibre takes place in the same manner as the contraction in the physiological sense, which had been observed in muscle fibres, i. e., the fibre thickens in direct proportion to the extent of the shortening. With thermal contraction, the contraction and relaxation processes seem to take place in exactly the same degree in a 40 per cent KI solution at 20 °C. This latter phenomenon we have termed chemical contraction-relaxation.

During both thermal and chemical contraction work is performed by the fibres inasmuch as they lift lead shot weighing from 80 to 100 times their own weight.

According to our submicroscopic findings the native collagen fibres separate into two phases during contraction and, besides, during relaxation the fibre undergoes transformation by losing the stabilizing substance of its intrinsic double refraction. It has been assumed that in contraction-relaxation one of the substances responsible for the double refraction of the collagen fibre dissolves. The investigations were therefore extended to inquiries into the amount of protein dissolving during both the thermal and the chemical contraction-relaxation processes. It has been found that the setting in of contraction and the dissolution of larger amounts of protein are not linked together. Only 1 or 2 per cent of the substance dissolve during heat contraction, and this tallies well with the observations of BANGA [1] and BANGA and BALÓ [2] that during the thermal contraction of the collagen a part of the mucoid in it goes into solution. On the other hand, dissolution of the protein starts in the course of the relaxation following contraction, a phenomenon which can be brought into agreement with the picture seen in the microscope. Separation into two phases sets in during contraction : there is an external homogeneous, and an

internal spiral part, as has been described in this paper when discussing our submicroscopic observations. With the beginning of relaxation the internal spiral part gradually disappears, and by the time relaxation is complete the collagen fibre is perfectly homogeneous. During this process from 10 to 15 per cent of the total protein in the collagen fibre is dissolved in 120 secs, at 67 °C. From the beginning of the relaxation to its completion an additional amount of protein (43 per cent) goes into solution. On further heating, the fibres slowly continue to dissolve. The results of this experiment are summarized in Table I.

Table I

Protein dissolution in native collagen fibres during thermal contraction and relaxation

Quantity of native collagen fibre in mg	H_2O in ml	Temperature °C	Period of contraction and relaxation, resp. sec.	State	Dissolution of collagen protein per cent
20	2	20	0	no change	0
20	2	20	0	no change	0
20	2	67	40	contraction	1,1
20	2	67	80	marked contraction	10
20	2	67	120	relaxation starts	15
20	2	67	300	relaxation	43
20	2	67	600	relaxation	73

Table II

Dissolution of native collagen fibre during chemical relaxation

Quantity of native collagen fibre in mg	40 per cent KI solution ml	Incubation time = period of contraction and relaxation, respectively, at 20 °C min.	State	Dissolution of collagen protein per cent
20	2	2	contraction	0
20	2	5	marked contraction	8
20	2	15	incipient relaxation	36
20	2	60	relaxation	75

The data presented in Table II show that the experiment concerned with protein dissolution during chemical contraction-relaxation in 40 per cent KI solution yielded similar, though somewhat less trenchant, results. The process is here a slow one, and washing the fibres, removed from the 40 per cent KI solution,

with water and alcohol may involve losses ; it is probably due to this methodological deficiency that more protein seems to be dissolving during chemical than thermal contraction. The results nonetheless confirm our assumption that there is no beginning of relaxation until the helical part visible in the submicroscopic structure of the fibres has vanished, which is a phenomenon associated with the dissolution of about 20 per cent of the total protein. The relaxed fibre is identical with the metacollagen fibre which binds a great amount of water and dissolves slowly on prolonged heating. The dissolved 20 per cent of the protein correspond to procollagen which in an independent experiment we have shown to gelatinize completely at this degree of heat. Accordingly, the contraction-relaxation process is accompanied by a loosening in the collagen fibre of the chemical bond between the procollagen and the metacollagen, and the procollagen in the fibres dissolves until relaxation is complete. This is the only explanation that can be offered for the observation that the contraction-relaxation process of the collagen fibres remains reversible if it is interrupted in the contractile phase. Whether we have to do with thermal or chemical contraction, the procedure in these instances is to remove the contracted fibre from the fluid, put it in cold water, stretch it to its original length, then wash it with alcohol and dry it. With fibres handled in this manner contraction again occurs, and this confirms the reversibility of the process. Theoretically there is no limit to reversibility, but in fact the process cannot be repeated more than 10 to 12 times. The reason for this is that during contraction there always begins a relaxation of limited extent accompanied by the dissolution of a little procollagen, and this prevents the process from running exactly the same course as the original process. For, after complete relaxation the fibre is no longer capable of contraction ; it no longer contains procollagen, whereas no contraction of the collagen fibre can take place unless in the combined presence of procollagen and metacollagen.

Experiments with elastase to prove procollagen and metacollagen to be different proteins

On combining the different phases of the contraction-relaxation process with hydrolysis with elastase, it is found that in the strictly contractile state only that part of the collagen is dissolved by the enzyme which in Fig. 2 appears to be homogenous (external phase). After treatment with elastase the picture is that in Fig. 6. This means that in the contractile phase where the two proteins are already separated, that is, the chemical bonds between procollagen and metacollagen has been loosened, the elastase is capable of dissolving the apparently isotropic part but still unable to dissolve the internal spiral part. Quantitative enzymatic investigations produced exactly the results which

on the ground of the microscopical observations they were excepted to yield. Quantitative dissolution experiments with a substantial quantity of fibres, in which contraction was induced in part thermally, in part chemically, have shown that the amount of protein dissolved by the enzyme has close bearings on the condition imparted to the fibres through the treatment. With the aid of elastase dissolution it is possible to draw a sharp line between the contractile and the relaxant phases. Whilst the collagen is in contraction only, not more than about 20 per cent of the protein dissolve from it upon the action of elastase ; the protein dissolved is the procollagen. The same protein dissolves during transition from the contracted phase to the relaxed phase. But for this, additional thermal energy, or, in the case of KI, chemical energy is required. In collagen, that what with thermal treatment at 67 °C is brought about in 100 sec, ensues upon the action of elastase at 37 °C in from 10 to 15 min. But even through longer incubation in elastase it is not possible to have more protein dissolved from a contracted fibre than its procollagen component, i. e. 20 per cent of its total protein content. The data presented in Table III show the interrelation of dissolution in elastase and the contraction-relaxation phase.

Table III

Interrelation between dissolution by elastase and the contraction and relaxation phases

Quantity of native collagen fibre in mg	Elastase 15 min. incub. at 37 °C mg	Contraction and relaxation phase, respectively	Dissolution by elastase per cent
20	0,5	no change	0
20	0,5	contraction	20
20	0,5	marked contraction	20
20	0,5	relaxation starts	70
20	0,5	complete elongation	100

In the incipient stage of relaxation, after the procollagen has dissolved, protein dissolution during elastase treatment is a direct and precise function of the extent of the relaxation. As long as the elongation of the relaxing fibres is 50 to 70 per cent of the original length, dissolution by elastase during incubation for 10 to 15 min. remains incomplete. But as soon as relaxation is completed it is a matter of minutes for the dissolution to be 100 per cent. Table III demonstrates the experimental results at which in the various phases of the contraction-relaxation process the thermal and chemical treatment, respectively, has been interrupted, and the amount of collagen dissolved by elastase studied.

As can be seen from the data in Table III, as long as the fibres are in a state of contraction, only 20 per cent of the protein are dissolved. Beginning relaxation is accompanied by gradual dissolution of all the collagen protein.

In the collagen fibres of the Achilles tendon of cattle, thermal contraction-relaxation is a slower process than in those of the rat tail tendon. The data in Table IV show the relation between contraction-relaxation of the Achilles

Table IV

Elicitation at different temperatures of contraction-relaxation phases in Achilles tendons and the relations of these phases to collagenolysis

Temp. °C	Incub. min.	Contraction-relaxation phase	Collagenolysis dissolution at 37 °C per cent	Period incubation with enzyme
55	10	no change	0	24h
60	10	no change	0	24h
67	5	60% contraction	17	24h
67	10	65% contraction	18	24h
70	5	incipient relaxation	70	3h
70	10	complete relaxation	100	20 min.

tendon and collagenolysis consequent upon the action of elastase. According to these data, there is no contraction at 55° or 60 °C and no dissolution of the pretreated fibres by elastase. The critical temperature at which the fibres begin to contract, is 67 °C. On keeping the collagen fibres at this temperature for 5 minutes, or even less, 20 per cent of their total protein contents dissolve when they pass into the contracted state. No matter how long the contracted fibres are incubated in elastase, more than 20 per cent will not dissolve. Relaxation once started, dissolution by elastase too begins again, and as soon as the fibres have become perfectly relaxed they are dissolved in elastase within minutes.

Discussion

Thermal contraction of collagen fibres is a phenomenon that has been known for long, but the fact that upon continued action of heat contraction is necessarily followed by relaxation has escaped the attention of investigators. Still, the two processes, contraction and the relaxation that follows it, have close bearings on the submicroscopic structure of collagen fibres. For this very reason this system presents a most suitable object for the study of function and structure. However, thermal contraction and relaxation are running a very rapid course and, therefore, difficult to differentiate for the purposes of chemical

analysis. On the other hand, chemical contraction-relaxation proceeds slowy at a low temperature, and the reaction can be interrupted in any phase at will and it becomes possible conveniently to study the submicroscopical and biochemical changes taking place during functioning. It was PARTRIDGE (1948) who reported that, apart from thermal contraction, the collagen fibres contract upon the action of certain chemical substances also at a moderate temperature (20 °C), but we have been the first to describe (1954) that contraction is followed by relaxation and that the two processes are interrelated. For the study of these phenomenon a 40 per cent potassium iodide solution proved to be most suitable. In all the other substances tested contraction-relaxation was slower and imperfect. Contraction is linked up with a very characteristic submicroscopic change. The native fibre, which submicroscopically appears to be perfectly homogenous, separates during contraction into two parts of different double refraction. In the presence of elastase the part of low birefringence differs in behaviour from the spiral part ; the former is dissolved by the enzyme, the latter does not. This proves that during contraction the collagen fibre decomposes into two kinds of protein ; this is submicroscopically observable and enzymologically confirmable. Relaxation arises on transmission of additional thermal or chemical energy. During relaxation the spiral structure is lost, submicroscopically a structure of low double refraction is left over, which is apparently homogenous. On the evidence of biochemical examinations, during the process from contraction to relaxation a protein (procollagen), making up about 20 per cent of the total protein is dissolved, and the residual relaxed fibre is what we call metacollagen. By its submicroscopic structure the relaxed fibre is a system in isotropic, and the stretched fibre one in anisotropic phase. From the point of view of colloid chemistry the native and the relaxed fibre differ from each other in quality. While the former swells but moderately in water, the latter is capable of exceedingly marked neutral swelling. In our previous paper [4] we described another way of preparing metacollagen, namely, by extracting the procollagen from the native fibre by means of a pH 4 citrate or acetate buffer, when a protein similar in composition to the relaxed fibre is left over. Yet this is not quite identical with the relaxed fibre for it still contains mucoid. The metacollagen fibre is no longer capable of thermal and chemical contraction but it is elastic. Upon the action of heat it fails to contract anisodiametrically; instead, it displays isodiametric shrinkage to the same extent in every direction, and this corresponds to the phenomenon of synaeresis BANGA, [5]. Function, i. e., contraction-relaxation, is linked up precisely with the structure of intact fibre. In the case of the collagen fibre the procollagen and the metacollagen are coupled chemically in the native fibre and this is the system which is capable to perform the function termed contraction-relaxation. Contraction is still reversible, because the bond between the two proteins is only loosened but not yet split. Relaxation, on the other

hand, is an irreversible process, understandable on the evidence of the biochemical analysis to the effect that during relaxation procollagen is extracted from the fibres. Thus, from the relaxed fibre the component is missing which is a fundamental constituent of the functioning fibre.

In respect of its function and structure, the collagen fibre can be brought into relation with the contractile element of muscle, actomyosin. On separating actin from myosin their ability of function cease. In the same way, if in the collagen the procollagen and the metacollagen are separated from each other, it is unable to continue contracting and relaxing. Accordingly, actomyosin as well as native collagen are to be regarded as biological and functional units which biochemically are built up of two structural proteins. The latter may dissociate, but then they are no longer capable of functioning. Only if coupled by chemical linkage to a single homogeneous protein, can they give rise to a functional unit.

Summary

1. The submicroscopic structure of collagen fibre has been studied under the polarization microscope. Thermal and chemical contraction-relaxation have been found to be suitable means by which to establish relations between the submicroscopic structure evidenced in these processes and the chemical composition of collagen fibres.

2. The contracted collagen fibre separates in two phases of different double refraction. Morphological separation is associated with the loosening of the chemical bond between the two structural proteins, procollagen and metacollagen, that build up the collagen fibre.

3. During thermal and chemical relaxation the picture in the polarization microscope represents a system which optically is in an isotropic phase and no longer capable of contraction, responding with synaeresis to the effect of heat. This system presents elasticity and is completely dissolved by elastase.

4. The investigations appear to prove our assumption that, like actomyosin, the native collagen fibre consists of two principal structural proteins (scleroproteins), procollagen and metacollagen.

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СУБМИКРОСКОПИЧЕСКАЯ СТРУКТУРА КОЛЛАГЕННЫХ ВОЛОКОН

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1. Авторы исследовали изменения структуры коллагенных волокон с помощью поляризационного микроскопа. Тепловое и химическое сокращение и расслабление оказались пригодными для выявления связей между субмикроскопической структурой, проявляющейся на основе этих процессов, и химическим построением коллагенных волокон.

2. Контрагированные коллагенные волокна отделяются на две фазы с различным двойным преломлением. Морфологическое отделение сопровождается расщеплением химической связи между двумя структурными белками, построящими коллагенное волокно, — проколлагеном и метаколлагеном. Это можно энзиматически доказать, так как из контрагированного волокна растворяются на действие эластазы 20% белка, в противоположность нативному, энзиматически нерастворимому волокну.

3. В ходе тепловой и химической реляксации поляризационномикроскопическая картина представляет систему, находящуюся с оптической точки зрения в изотропной фазе, которая больше не способна к сокращению и отвечает на тепловое воздействие явлением синереза. Эта система совершенно растворяется эластазой.

4. Исследования подтвердили то предположение авторов, согласно которому нативное коллагенное волокно — подобно актомиозину — построено из двух главных компонентов структурного белка (склеропротеизы) — из проколлагена и метаколлагена.

SUBMIKROSKOPISCHE STRUKTUR VON KOLLAGENFASERN

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1. Die Veränderungen der submikroskopischen Struktur der Kollagenfasern wurden polarisations-mikroskopisch untersucht. Die Wärme- und chemische Kontraktion-Relaxation erwies sich zur Untersuchung der Zusammenhänge zwischen der mit diesem Verfahren sichtbar gemachten submikroskopischen Struktur und dem chemischen Aufbau der Kollagenfasern als geeignet.

2. Die kontrahierten Kollagenfasern weisen zwei Phasen mit verschiedener Doppelbrechnung auf. Die morphologische Differenzierung wird von der Lockerung der chemischen Bindungen zwischen den beiden Struktureiweissen, dem Prokollagen und Metakollagen begleitet. Dies ist enzymatisch nachweisbar, da aus der kontrahierten Faser auf Wirkung von Elastase 20% Eiweiss gelöst wird, gegenüber der nativen enzymatisch unlösbaren Faser.

3. Während der Wärme- und chemischen Relaxation stellt das im Polarisationsmikroskop sichtbare Bild optisch ein isotropes System dar, das zur Kontraktion nicht mehr fähig ist, und auf Wärmeeinwirkung mit Synerese reagiert und wird durch Elastase restlos gelöst.

4. Die Befunde bewiesen die Annahme, dass die nativen Kollagenfasern — ähnlich dem Aktomyosin — aus zwei Struktureiweissen (Skleroproteiden) — dem Prokollagen und Metakollagen — bestehen.

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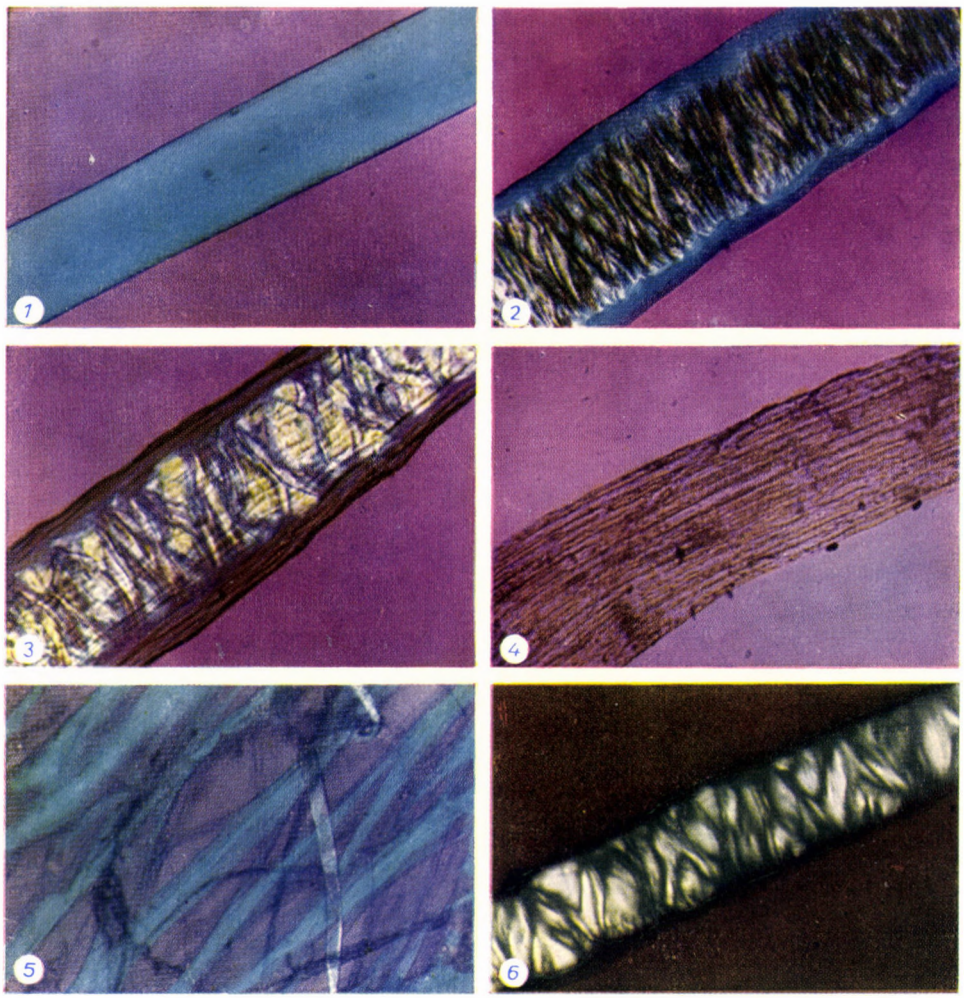

Fig. 1. Native collagen fibre under the polarization microscope, showing intensive positive double refraction in polarized light

Fig. 2. Contracted collagen fibre under the polarization microscope, showing its separation in two phases displaying double refraction of different intensity

Fig. 3. The same as Fig. 2. in a more advanced state of contraction. While in Fig. 2 double refraction of the external part is only decreased in relation to that of the internal part, here it has vanished completely

Fig. 4. The metacollagen in isotopic phase fails to display double refraction under the polarizing microscope.

Fig. 5. After pretreatment with pH 4 citrate buffer the fibre can be broken up into filaments on limited mechanical action

Fig. 6. The part of the contracted fibre visible in Fig. 3 and displaying no double refraction (metacollagen is dissolved on the action of elastase)

(Figs. 1 to 5 prepared with the insertion of crossed nicols and gypsum I lamella)