A STUDY OF THE CHEMICAL SHRINKAGE AND RELAXATION OF COLLAGEN FIBERS

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SUMMARY

The chemical contraction and relaxation of collagen fibres are described in terms of chemical kinetics, and the factors influencing the course of fibre length variations are studied. The course of chemical contraction and relaxation is investigated both by measuring the fibre length and by determining the concentration of the liberated substances. The influence of the pH of the medium and that of reducing agents upon the shape of the curves showing the dependence of fibre length on time is studied.

INTRODUCTION

Though the thermal contraction and relaxation of collagen fibres have been thoroughly investigated[1], comparatively little attention has been paid to the so-called chemical contraction and relaxation[2–4]. The majority of papers[1,5] in this field has been restricted to the study of the influence of electrolytes and non-electrolytes on the shrinkage temperature of the collagen fibre, and in this connection it has been pointed out that perchlorate, thiocyanate and iodide ions lower the shrinkage temperature markedly.

BANGA, BALÓ AND SZABÓ[3–4] studied the changes of collagen fibres in 40 % potassium iodide and showed that the shrinkage of the fibre is connected with the splitting-off of a mucoproteinic substance and that relaxation is due to the rupture of the linkage between procollagen and metacollagen. Of interest is the finding by these authors that reducing substances acting in acid medium effect "rejuvenation" of the reactivity of the fibres and that, inversely, oxidizing agents bring about ageing of collagenous structures. They further showed that the course of contraction and relaxation materially depends on the age of the organism.

In applying this method to the study of some problems of pathophysiological character, however, we were carrying out a fundamental investigation of the individual factors which could act in chemical contraction and relaxation, as well as giving a description of the actual course of fibre length variations in terms of chemical kinetics. After studying these principal questions we were further occupied with the problem of a possible "rejuvenation" of the reactivity of collagen fibres in the sense described by the Hungarian authors[3–4].

THEORETICAL

We shall try to find an expression describing the dependence of the total fibre length l on time for the case of chemical shrinkage and relaxation of the collagen fibre, the course of which together with the notation of the quantities involved is given in Fig. 1.

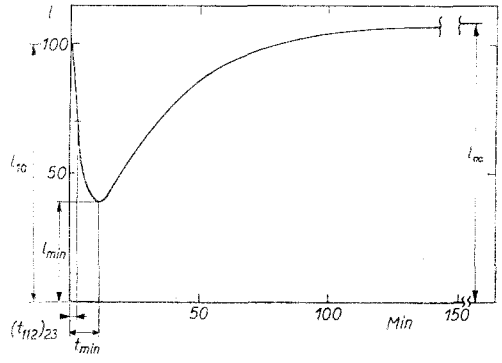

Fig. 1. Typical course of a model curve of chemical contraction and relaxation of collagen fibres. Nomenclature: l (fiber length), t (time), $l_{1,0}$ (fibre length at the time $t = 0$), l_{min} (minimum fibre length), l_∞ (extreme fibre length), $(t_{1/2})_{23}$ or t_{min} (time required for attainment of half the reduction of the initial fibre length or of maximum shrinkage respectively). Parameters of the curve: $k_{12} = 0.20$; $k_{23} = 2.00$; $k_{34} = 0.345$; $l_1 = 100$; $\varphi_2 = 1.0$; $\varphi_3 = 0.1$; $\varphi_4 = 1.083$.

Let us assume that the total length of the collagen fibre l at an arbitrary time is given by the sum of the lengths l_i of the fibre-forming components

$$l = \sum_i l_i \tag{1}$$

The minimum on the graph of the function (Fig. 1) as well as the relative positions of this minimum and the points of inflection indicate that at least three changes participate in the process of shrinkage and relaxation. From the shape of the graph of the function we conclude that it expresses a consecutive reaction, which can be described by the scheme

$$A_1 \xrightarrow{k_{12}} A_2 \xrightarrow{k_{23}} A_3 \xrightarrow{k_{34}} A_4 \tag{2}$$

We assume that the reactions in scheme (2) are irreversible reactions of the first order, which assumption appears to be justified with regard to experimental data.

The kinetic scheme (2) was studied by RAKOWSKI[6], who expressed the concentration of the components A_i as explicit functions of time.

Acting on the assumption that the "concentration" of the component A_i in the fibre under investigation is linearly proportional to its length l_i, we can represent the dependence of the length l_i ($i = 1, 2, 3, 4$) on time by

$$l_1 = l_{1,0} \exp(-k_{12}t) \tag{3}$$

$$l_2 = \varphi_2 \frac{l_{1,0}\, k_{12}}{k_{23} - k_{12}} (\exp[-k_{12}t] - \exp[-k_{23}t]) \tag{4}$$

$$l_3 = \varphi_3 l_{1,0} k_{12} k_{23} \left\{ \frac{\exp(-k_{12}t)}{(k_{23}-k_{12})(k_{34}-k_{12})} - \frac{\exp(-k_{23}t)}{(k_{23}-k_{12})(k_{34}-k_{23})} + \frac{\exp(-k_{34}t)}{(k_{34}-k_{23})(k_{34}-k_{12})} \right\} \tag{5}$$

$$l_4 = \varphi_4 l_{1,0} \left\{ 1 - \frac{k_{23}k_{34} \exp(-k_{12}t)}{(k_{23}-k_{12})(k_{34}-k_{12})} + \frac{k_{12}k_{34} \exp(-k_{23}t)}{(k_{23}-k_{12})(k_{34}-k_{23})} - \frac{k_{12}k_{23} \exp(-k_{34}t)}{(k_{34}-k_{23})(k_{34}-k_{12})} \right\} \tag{6}$$

In eqns. (3)–(6) k_{ij} denotes the first-order rate constant of the conversion of A_i to A_j, l_1 is the length of the component A_1 at time t, $l_{1,0}$ represents the length of the fibre A_1 at time $t = 0$, φ_i are the relative lengths of the components A_i, defined by

$$\varphi_i = l_{i,0}/l_{1,0} \; (i = 1,2,3,4) \tag{7}$$

The values $l_{i,0}$ ($i = 2, 3, 4$) denote the length of the fibre A_2, A_3, A_4, which is equivalent to the length of the fibre A_1 at time $t = 0$, hence $A_{1,0}$.

For the cases in which we are interested, the dimensionless parameters satisfy the inequalities

$$\varphi_2 \overset{<}{=} 1 \tag{8}$$

$$0 < \varphi_3 < 1 \tag{9}$$

$$\varphi_4 \overset{\leq}{=} 1 \tag{10}$$

The total fibre length l, accessible to measurement, is according to eqn. (1) given by the sum of eqns. (3)–(6). Thus we obtain eqn. (11)

$$l = \sum_{i=1}^{4} l_i = K_1 \exp(-k_{12}t) + K_2 \exp(-k_{23}t) + K_3 \exp(-k_{34}t) + l_{1,0}\varphi_4 \tag{11}$$

The constants K_i are defined by

$$K_1 = \frac{l_{1.0}}{(k_{23}-k_{12})(k_{34}-k_{12})}\{(k_{23}-k_{12})(k_{34}-k_{12}) + \varphi_2 k_{12}(k_{34}-k_{12}) + \varphi_3 k_{12}k_{23} - \varphi_4 k_{24}k_{34}\} \tag{12}$$

$$K_2 = \frac{l_{1,0}}{(k_{23}-k_{12})(k_{34}-k_{23})}\{-\varphi_2 k_{12}(k_{34}-k_{23}) - \varphi_3 k_{12}k_{23} + \varphi_4 k_{12}k_{34}\} \tag{13}$$

$$K_3 = \frac{l_{1.0}k_{12}k_{23}}{(k_{34}-k_{23})(k_{34}-k_{12})}\{\varphi_3 - \varphi_4\} \tag{14}$$

The magnitudes appearing in eqns. (12)–(14) have already been defined. From the first and second derivatives of the total length l (eqn. (11)) with respect to time we can calculate the conditions for the minimum and the point of inflection of the function. Practically, however, the respective relations are hardly usable because of the complexity of the summary constants they contain.

The determination of the rate constants k_{12}, k_{23} and k_{34} (eqn. (11)) from the known time-dependent course of shrinkage and relaxation is a very intricate task, admitting no exact solution at all. In the investigated cases, however, the rate constants k_{12} and k_{23} are always sufficiently large in comparison with the rate constant k_{34}. Therefore, it is possible for sufficiently large time values to neglect in equation (11) the terms involving the exponential curves $e^{-k_{12}t}$ and $e^{-k_{23}t}$ beside the other terms. Thus equation (15) is satisfied with sufficient accuracy:

$$l = K_3 \exp(-k_{34}t) + l_{1,0}\varphi_4 \tag{15}$$

By rearrangement and taking the logarithm of equation (15) we obtain

$$\lg(l - l_{1,0}\varphi_4) = \lg K_3 - k_{34}t \tag{16}$$

Equation (16) defines a straight line, the slope of which gives the rate constant k_{34}. Unfortunately, the exact expressions from which the rate constants k_{12} and k_{23} could be ascertained are too complicated. For this reason, we approximately replace the

constant k_{23} by half the time $(t_{1/2})_{23}$ required for attainment of the minimum fibre length (l_{min}), which is a value roughly inversely proportional to the constant k_{23} sought.

Finally, it should be noted that by means of the criterion suggested by VRIENS[7] for a reaction of the type $A_1 \rightarrow A_2 \rightarrow A_3$ one can be certain that the shrinkage and relaxation of collagen fibres proceed according to the scheme of a consecutive and not a side reaction. If, namely, the ratio of the concentration of the split-off constituent causing shrinkage to the concentration of the substance causing relaxation in the initial phase of the reaction is approximately constant, the scheme of side reactions predominates, and, inversely, if this ratio materially diminishes with time (which applies in our case), the scheme of the consecutive reaction becomes manifest.

Finally, it may be added that in special cases the three-stage scheme considered (2) becomes a two-stage scheme, and then it is a simple matter to determine the rate constants.

METHODS

The collagen fibres were isolated either from the tails of living, anaesthetized rats, or from tails cut off in such a way that the skin was left on the preparation and the fibre was pulled out through a cut of about 0.5 cm in length at a distance of 2 cm from the tail end. Since we did not always succeed in pulling out a single fibre, the extracted fibres were disintegrated as quickly as possible into elementary fibrils, that were further indivisable mechanically; after isolation these were immediately clamped into an indented rubber stopper. The free end of the fibre was at a certain distance pressed into a lead foil weighing 0.050 g, unless otherwise stated. The suspended fibre was transferred with the respective solution to a test tube of 14 × 1.4 cm, placed in a water thermostat maintaining the prescribed temperature with an accuracy of ± 0.1°. The experiments were carried out with collagen fibres from adult rats; the diameter of the fibres was from 0.14 to 0.15 mm.

Experimentally it was found that the error in determining the rate constant (k_{34}) under the usual conditions amounted to ± 10 %, the error in ascertaining the time required to attain the minimum length (t_{min}) was about ± 15 %, and the deviations of the other magnitudes varied by about ± 5 %. Experiments with fibres of 2–10 cm in length showed that the uncertainty in fibre length manifesting itself in routine tests involves a negligible error in the determination of the quantity under investigation.

The influence of the magnitude of the weight (0.050–0.150 g) is apparent from Table I.

TABLE I

INFLUENCE OF THE MAGNITUDE OF THE LOAD (m) ON THE COURSE OF SHRINKAGE AND RELAXATION OF COLLAGEN FIBRES FROM A RAT WEIGHING 135 g

2.5 M potassium iodide, 24.0°, initial fibre length 6.0 cm.

m (g)	l_{min} (cm)	l_∞ (cm)	t_{min} (min)	$10^2 \cdot k_{34}$ (min^{-1})	φ_∞	φ_{min}
0.050	3.0	5.4	3.0	5.0	0.90	0.50
0.100	3.1	5.8	3.5	4.7	0.97	0.52
0.150	3.5	6.55	3.3	3.3	1.09	0.58

RESULTS AND DISCUSSION

Measurement of total fibre length and of concentration of the split-off substances

In the investigation of the substances split-off in the course of shrinkage and relaxation of collagen fibres in 2.5 M $NaClO_4$ the experiment was carried out as follows. About 150 fibres, isolated by the above method, were transferred to a solution of 2.5 M $NaClO_4$ and in the course of shrinkage and relaxation specimens of the solution were withdrawn to measure their light absorption at 275 mμ (absorption corresponding to amino acids with aromatic nuclei in non-collagenous proteins), and after acid hydrolysis of these specimens (6 N HCl, 140°, 4 h in sealed test tubes) the hydroxyproline content was determined according to NEUMAN AND LOGAN[8] and that of proline according to CHINARD[9].

Fig. 2 illustrates the dependence of these quantities on time, the individual curves (1, 2, 3) being superimposed on the diagrams of the exponential functions. From this figure it is evident that a simple exponential curve covers the course of the experimental values for the time beginning at $t = 3$ min. During the first three minutes the reaction proceeds relatively very rapidly and evidently exponentially too (Fig. 3). On the course of the variations of the absorption (for $t > 3$ min) at 275 mμ (Curve 2, Fig. 2) is superimposed the exponential curve for the value of the rate constant $k_{23} = 2.26 \cdot 10^{-2}$ min^{-1}. On the course of the concentrations of

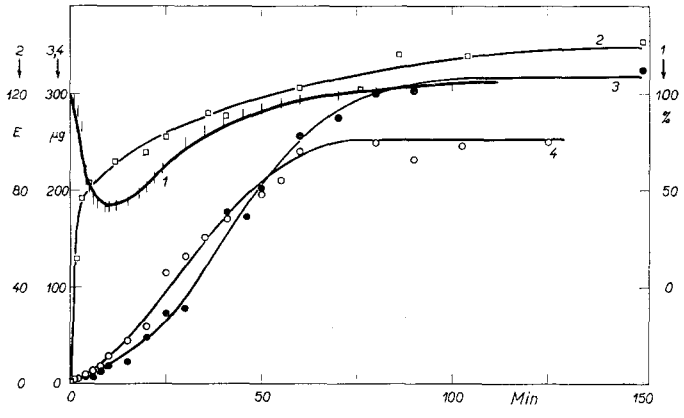

Fig. 2. Course of the reaction of the collagen fibre as observed by measurement of the total fibre length and of the concentration of the split-off substances. The total fibre length in cm (1), extinction of the substance with an absorption maximum at 275 mμ (2) and the concentrations of proline (3) and hydroxyproline (4) in the hydrolysates are plotted against time. At curve 1 the theoretical curve (eqn. (11)) is laid through experimental points for the constants: $k_{12} = 0.19$; $k_{23} = 1.9$; $k_{34} = 0.04$; $\psi_2 = 1.0$; $\psi_3 = 0.1$; $\varphi_4 = 1.083$. Data concerning the other curves are given in the text.

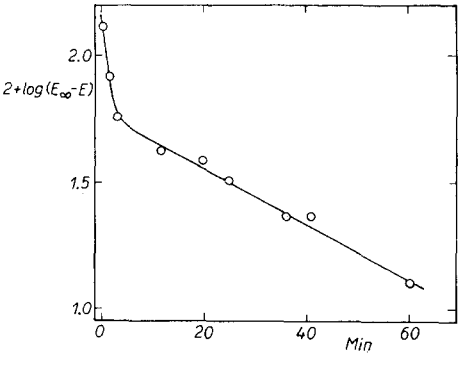

Fig. 3. Course of the splitting-off of the substance with an absorption maximum at 275 mμ plotted against time in semilogarithmic coordinates.

hydroxyproline (Curve 4, Fig. 2) and proline (Curve 3, Fig. 2) are superimposed empirical exponential curves.

Through experimentally ascertained points indicating the dependence of the total fibre length on time (Curve 1, Fig. 2) is laid a theoretical curve whose constants k_{34} and k_{23} were estimated from the course of the splitting-off of procollagen and mucoprotein, respectively. The value φ_4 necessary for the calculation was taken from experimental data on Curve 1; the values φ_2, φ_3 and k_{12} were determined by stepwise approximation.

On some fibres from the group which served for the experiment just described the course of the reaction was also studied by measuring the length, *i.e.* by the method currently used in this work. Curve 1 in Fig. 2 represents the course of shrinkage and relaxation; through experimentally ascertained points a theoretical curve is laid based on the rate constants resulting from the investigation of the concentration of proline and hydroxyproline and from the determination of the light absorption at 275 mμ (Fig. 2).

From the results obtained it follows that the fastest process in the chemical contraction and relaxation of the collagen fibre is the splitting-off of a substance exhibiting an absorption maximum at 275 mμ and containing no hydroxyproline. As far as time is concerned, the phase of the contraction of the collagen fibre corresponds to this reaction.

From Fig. 3 it is apparent that the splitting-off of the mucoproteins (the character of these substances was investigated by PARTRIDGE[10], JACKSON[11], and BANGA AND BALÓ[12]) from the fibre takes place in two phases. With regard to the considerable velocity of the first phase, we believe that here the mucoprotein of the ground substance, adhering to the surface of the collagen fibre, is concerned, and that only in the second phase does penetration of the electrolyte into the fibrous structure and gradual loosening of the fibre material occur; the principal constituent of this material is the substance that exhibits an absorption maximum at 275 mμ.

The phase of splitting off the mucoproteinous substance is followed by the liberation of a material containing hydroxyproline and proline in the ratio of approximately 1:1. At the time when the collagen fibre relaxes and attains its final extreme length practically all protein of the collagenous type corresponding to procollagen[3-4], removable under given conditions, is split off.

Influence of the composition of the solution upon the course of the shrinkage and relaxation curves of collagen fibres

In the systematic investigation of the anions and cations producing chemical shrinkage and relaxation it was found that, in addition to 2.5 M potassium iodide[2-4], solutions of other salts with the same concentration (Table II) exhibit qualitatively and quantitatively a similar effect. Apart from pseudo-halides (thiocyanate, xanthogenate) perchlorate, too, has proved to be effective. It is known that these highly deformable anions constitute the end of Hofmeister's series of lyotropic anions:

$$SO_4^{2-},\ C_2H_3O_2^-,\ Cl^-,\ Br^-,\ NO_3^-,\ ClO_3^-,\ I^-,\ SCN^-$$

Besides these, our examination was extended to a number of salts (sodium cyanide, ammonium acetate, lithium chloride, sodium bromide, potassium nitrate, potassium chloride), which in 2.5 M solution did not produce any changes in the

TABLE II

INFLUENCE OF THE ANION COMPOSITION ON SHRINKAGE AND RELAXATION OF COLLAGEN FIBRES FROM A RAT WEIGHING 150 g

2.5 M solutions of sodium and potassium salts, 24.0°, load 0.150 g, initial fibre length 6 cm.

Salt	l_{min} *(cm)*	l_∞ *(cm)*	$(t_{1/2})_{23}$ *(min)*	t_{min} *(min)*	$10^2 \cdot k_{34}$ *(min^{-1})*	φ_4	φ_{min}
Sodium iodide	3.3	6.2_5	3.0	9.0	3.6	1.04	0.55
Potassium thiocyanate	3.3	6.8_7	1.0	2.0	2.8	1.14	0.55
Potassium ethylxanthogenate	2.25	8	0.5	2.0	5	1.3	0.38
Sodium perchlorate	3.0_7	6.4_5	2.0	5.5	2.5	1.08	0.51

length of collagen fibres within 180 min. Also ineffective were 5 M solutions of sodium chloride and sodium nitrate. Since sodium perchlorate is in the group of effective salts most stable towards oxidizing and reducing agents and most easily obtainable in a pure, defined state, most of the further experiments were carried out with this salt.

In the series of alkali iodides (from lithium up to caesium) we found that under the conditions described the cation exerts no influence on the curves of shrinkage and relaxation.

Influence of the concentration of sodium perchlorate solutions and of the ionic strength of the reaction medium

From the data given in Table III it is apparent that up to a concentration of 1.9 M $NaClO_4$ no change in the fibre length was observed within 120 min. At concentrations from 2 M up to 3.5 M, the fibres immersed in these solutions exhibit the typical course of shrinkage and relaxation. In these cases it can be observed (Table III) that the half-period of the shrinkage phase (splitting-off of mucoprotein) significantly diminishes with increasing electrolyte concentration. For the very high rise in the reactivity at the step from 1.9 M to 2.0 M sodium perchlorate solution we have hitherto found no explanation. If we use the values $(t_{1/2})_{23}$ as first approximations

TABLE III

DEPENDENCE OF THE TIME COURSE OF SHRINKAGE AND RELAXATION OF COLLAGEN FIBRES FROM A RAT WEIGHING 120 g ON THE CONCENTRATION OF SODIUM PERCHLORATE SOLUTIONS

25.0°, load 0.050 g, initial fibre length 6.0 cm.

M $NaClO_4$	l_{min} *(cm)*	l_∞ *(cm)*	$(t_{1/2})_{23}$ *(min)*	t_{min} *(min)*	$10^2 \cdot k_{34}$ *(min^{-1})*	φ_4	φ_{min}
1.9	no changes within 120 min						
2.0	3.60	6.05	6	12.5	0.0385	1.01	0.60
2.1	3.55	6.20	4.5	3.0	0.02903	1.03	0.59
2.2	3.55	7.05	3.0	6.5	0.0289	1.17	0.59
2.3	3.50	6.85	2.0	4.5	0.0228	1.14	0.58
2.4	3.25	6.15	2.0	4.5	0.0253	1.025	0.54
2.5	3.50	6.65	3.0	8.0	0.0362	1.11	0.58
2.7_5	3.15	6.25	0.5	3.5	0.0325	1.04	0.52
3.0	2.85	6.2	1	3.5	0.0451	1.03	0.48
3.5	2.85	6.55	0.5	1.5	0.0322	1.09	0.48

for calculating the rate constants k_{23} of the splitting-off of mucoprotein and plot these rate constants against the molarity of sodium perchlorate, we obtain an approximately linear dependence. Hence it is evident that for the rate constant of the splitting-off of protein with an absorption maximum at 275 mμ, for a constant quantity of collagen fibres, eqn. (17) holds:

$$k_{23} = k\,[ClO_4^-] \tag{17}$$

where $[ClO_4^-]$ denotes the molarity of sodium perchlorate and k_{23} the corresponding rate constant. The suitability of this equation for the correlation of our data indicates that the splitting-off of mucoprotein (for $t > 3$ min) has the character of an ordinary chemical reaction.

Moreover, it was proved that a change in the ionic strength of the 2.5 M sodium perchlorate solution from the value of 2.5 M to 6.5 M (ionic strength adjusted with lithium chloride) does not bring about a significant change in the rate constant k_{34}. Changes of $(t_{1/2})_{23}$ are likewise not significant with regard to the experimental errors; only the magnitude of contraction rises with increasing ionic strength.

A striking feature in the evaluation of the influence of cations and anions upon the time-dependent course of shrinkage and relaxation of collagen fibres is the negligible effect of the composition of the cations in comparison with the pronounced effect of the anion composition. The effect of lithium, sodium and potassium iodides is practically the same. On the other hand, beginning with iodide, the anions forming the end of Hofmeister's series are effective. This relates to iodide, perchlorate, thiocyanate and xanthogenate. These relatively little solvated anions are known for their swelling effect. It may be assumed that shrinkage is due to a change in hydration and thus also to the character of the bonding forces of the protein molecules of the collagen fibre, as a result of the linking of the anions to these structures. With regard to the insignificant differences between the cations, it may further be concluded that the reaction causing shrinkage is probably due to sorption of the negatively charged, only slightly hydrated ion on the positively charged centre. On the whole, it can be stated that there are no significant differences in the efficiency of thiocyanate and perchlorate. Potassium ethyl xanthogenate is significantly more effective than other active anions in that both shrinkage and relaxation proceed essentially faster. However, we have observed that, in contrast to other reagents, the fibre ruptures before attaining its limiting length.

Dependence on temperature

In studying the dependence of shrinkage and relaxation on temperature we found that this dependence under the conditions applied, can be investigated only in a narrow temperature range. Excessive slowness or cessation of the process at lower temperatures and a very high rate at higher temperatures impede experimental investigation. This is obviously due to the comparatively high energy of activation of at least one of the rate processes.

As far as the relaxation process is concerned, we were able to study the dependence of the actual rate constants (k_{34}); the rate of the shrinkage process, on the other hand, we have characterized only by the value of the half-period $(t_{1/2})_{23}$, which, however, involves no change in the Arrhenius energy of activation (Table IV).

From the values of the energies of activation it is apparent that shrinkage in-

TABLE IV

ARRHENIUS ENERGY OF ACTIVATION OF THE SHRINKAGE AND RELAXATION PROCESS OF THE COLLAGEN FIBRE IN A 2.5 M SODIUM PERCHLORATE MEDIUM IN THE RANGE OF 16–30°

Process	*Rat weight (g)*	E^* *(kcal·mol^{-1})*
Shrinkage	290	57 ± 4
Shrinkage	240	53 ± 4
Relaxation	290	9.6 ± 1

volves a process which, as is to be inferred from the high values of the energy of activation, is accompanied by the disappearance of relatively very firm chemical bonds. We know that this process splits off mucopolysaccharide, and perhaps mucoprotein, from the collagen fibre. On the other hand, the splitting-off of procollagen is a process that sets in much more readily. Since the corresponding value of the energy of activation amounts to only 10 kcal·mol^{-1}, we conclude that the splitting-off of procollagen is connected with the disappearance of comparatively weak bonds.

Influence of reducing agents and the pH value of the medium on the shrinkage and relaxation curves

BANGA, BALÓ AND SZABÓ[4,12] reported that by the action of various reducing agents in acid medium collagen fibres from adult or old rats assume the properties of fibres isolated from younger organisms. The magnitude of shrinkage is here smaller and rapid relaxation sets in, attaining a greater length than the initial one.

With regard to this finding, we have studied the influence of 0.01 M solutions of a number of reducing agents on the course of chemical contraction and relaxation of collagen fibres. The reducing substances used were quinol, chromium dichloride, titanium trichloride, ferrous ammonium sulphate and ascorbic acid. The last-named substance had to be employed also in solutions of 0.001 M concentration.

Freshly isolated collagen fibres from rat tails were soaked for 30 min in solutions of these substances, and it was found that no significant variation of the length sets in and that the general appearance of the fibre changes characteristically[13,14]. The fibre was then slightly dried with filter paper and immediately transferred to a 2.5 M sodium perchlorate solution, and the dependence of the length on time investigated.

The results obtained (Table V) are not in agreement with the findings of the Hungarian authors. Among five reducing substances examined only the 0.001 M solution of ascorbic acid brought about a pronounced change of the contractibility of the fibre in the sense of "rejuvenation". The other substances, apart from titanium trichloride, caused a marked change in the course of shrinkage and relaxation, which according to BANGA, BALÓ AND SZABÓ[4] could be characterized as "ageing". Soaking in 0.01 M ascorbic acid for 30 min resulted in distinct swelling of the fibre, which became rubber-like and elastic and immediately extended when loaded with a weight of 0.050 g. For this reason, we used concentrations lower by one order of magnitude (0.001 M), in which a certain amount of swelling still took place, but the fibre could be further investigated in a solution of sodium perchlorate.

In order to find out whether the "rejuvenating" effect of ascorbic acid lies in the reducing properties of this substance or is due to the acidity of its solution, we

TABLE V

INFLUENCE OF PRECEDING ACTION (30 min) OF AQUEOUS SOLUTIONS OF REDUCING SUBSTANCES ON THE COURSE OF SHRINKAGE AND RELAXATION

2.5 *M* sodium perchlorate, 25.0°, load 0.050 g, initial fibre length 6 cm.

Reducing substance (0.01 M solutions)	*pH*	*Rat weight (g)*	l_{min} *(cm)*	l_∞ *(cm)*	$(t_{1/2})_{23}$ *(min)*	t_{min} *(min)*	$10^2 \cdot k_{34}$ *(min^{-1})*	φ_t	φ_{min}
Control fiber	—	130	3.5	6.13	2.5	7	3.98	1.02	0.583
Quinol	7.1	130	2.6	4.48	3	9	1.36	0.74	0.434
Chromium dichloride	5.3	130	3.6_2	5.28	7	17.5	2.35	0.94	0.608
Titanium trichloride	6.1	130	3.1_5	5.93	2	5.5	4.33	0.99	0.525
Ferrous ammonium sulphate	6.3	130	3.2	5.78	2	8	3.98	0.96	0.533
Ascorbic acid	2.6	250	5.9			3			
Ascorbic acid*	3.3	250	1.8	6.0	1	8	1.27	1.00	0.300
Fysiological solution	7.2	250	2.8	6.2	3	7.5	2.30	1.04	0.466

* 0.001 *M* solution.

TABLE VI

INFLUENCE OF PRECEDING ACTION (30 min) OF BRITTON–ROBINSON BUFFER ON THE COURSE OF SHRINKAGE AND RELAXATION

2.5 *M* sodium perchlorate, 25.0°, load 0.050 g, initial fibre length 6.0 cm.

pH	l_{min} *(cm)*	l_∞ *(cm)*	$(t_{1/2})_{23}$ *(min)*	t_{min} *(min)*	$10^2 \cdot k_{34}$ *(min^{-1})*	φ_t	φ_{min}
3.95	6.0	6.7	0.0	0.0	70*	1.11	1.00
4.8	5.8	6.3	0.5	2.0	20*	1.05	0.97
5.2	5.7	6.6	1.0	2.5	17	1.11	0.95
5.35	5.5	6.2	1.0	2.5	11*	1.03	0.88
5.55	5.4	6.4	1.0	3.0	7.7*	1.06	0.90
5.75	4.5	6.3	1.9	3.0	6.8	1.08	0.75
5.95	3.95	6.2	2.2	4.0	4.8	1.03	0.66
6.0	2.75			5.5	3.8		
6.75	2.85	6.05	3.2	8.0	3.4	1.01	0.48
7.5	2.3			9.5	2.9		
8.6	2.8	5.9	1.5	9.0	2.4	0.98	0.47
10.55	2.75	5.6	1.4	8.5	2.8	0.93	0.46
11.7	2.45	5.9	0.8	7.0	2.5	0.99	0.41

* Calculated from the half-period.

studied the influence of the pH value of the medium on the course of chemical contraction and relaxation of collagen. For this purpose we soaked collagen fibres from the same rat for 30 min in a series of buffers according to Britton and Robinson in the pH range from 4.0 to 11.7 and examined them further in the usual way. The results are summarized in Table VI and show unambiguously the importance of the acidity of the solution used for pretreatment of the collagen fibre. Within the pH range from 7.5 to 11.7 the changes of the reactivity of the fibres are insignificant. In acid solutions (pH below 7.0), however, a gradually increasing influence upon the course of shrinkage and subsequent relaxation becomes manifest, and, according to the publications[3–4] quoted, this effect could be termed "rejuvenation" of the collagenous structure.

If we plot the rate constant k_{34} against the pH value of the buffer in which

the fibres were previously incubated, we obtain a curve which in semilogarithmic coordinates shows two approximately linear parts. From Fig. 4 it is apparent that preliminary incubation of the fibres in buffers of pH 4–6 exerts a material influence on the rate of relaxation, whereas incubation in a medium of pH 7–11 is ineffective.

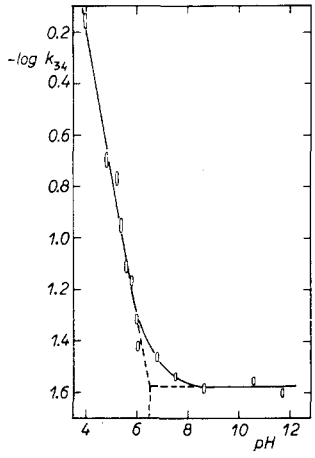

Fig. 4. Dependence of the rate constant k_{34} on the pH value of the Britton–Robinson buffer in semilogarithmic coordinates. In the figure are plotted the data of Table IV.

We conclude that the hydrogen ion concentration in buffers of pH 4–6 is sufficient for protonation of the basic groups in collagen and consequent structural changes, which manifest themselves, apart from other phenomena, also by a change in relaxability. It seems that the intersection of both linear parts of the graph of the dependence of log k_{34} on the pH value (Fig. 4), located at pH 6.5, corresponds to the isoelectric point of collagen, for which values most frequently given in the literature[13,14] range from 6 to 7.5.

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