V. THE SWELLING OF SINGLE COLLAGEN FIBRE-BUNDLES.

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KAYE AND JORDAN-LLOYD [1924] in an investigation of the swelling of fibrous tissue showed that single teased-out collagen fibres altered in length and breadth when treated in solutions of acids and alkalis. In that work single fibre-bundles were obtained and the length and breadth were determined under the microscope by means of an eyepiece micrometer. The fibre-bundles were then treated with various reagents and the fibre-bundle re-measured. This method of observation is, however, extremely tedious and it is complicated by the great difficulty of measuring the breadth of the fibre-bundle, since this can vary to a very marked degree along the length of the bundle. This trouble becomes almost insurmountable when the fibre-bundles are swollen in acid solution, since there is a large amount of distortion and any attempt to express the results numerically leads to large errors.

It was also shown that the swelling of hides and skins in the various liquors was dependent on the degree to which the hide fibres had been freed from each other. Thus the increase in volume of hide powder was of the same order as that of gelatin, while in the cases of pieces of hide and skin the percentage increase, although running parallel with that of hide powder, was very much smaller. The obvious difference between hide, as such, and hide powder is that in the former the individual fibres are not free to contract along their length nor are they free to increase in width since they are more or less closely interwoven. Thus the results obtained with loose fibre-bundles are not truly comparable with the effect produced on pieces of skin.

It was therefore decided to endeavour to follow more accurately the effect of various solutions on single collagen fibre-bundles which were lightly held by their ends so that the effects observed would be more comparable with those taking place in the fibres when hides and skins are treated with different reagents. The first experiments were directed towards following the swelling of the fibres in acid liquors of known p_H value.

EXPERIMENTAL.

In the preliminary experiments, single bundles of purified collagen (acetone-dried), obtained from ox hide, were teased out and fastened at the ends to a glass slide by means of paraffin wax. The fibre-bundle was then soaked in distilled water, which was afterwards replaced by $N/100$ hydrochloric acid. The fibre swelled in the acid but it was not distorted, neither was there any marked indication of the constricting elements of reticular tissue which encircle the fibres and which are generally to be observed when free single fibre-bundles are swollen in dilute hydrochloric acid. In a further experiment, an attempt was made to cement the fibre-bundle to two fine glass rods, one of which was fixed and the other suspended from a balance so that an increased load could be applied to the fibre-bundle. It was found that when there was no load on the fibre-bundle the characteristic contraction in length and increase in width which occurs when collagen fibres are treated with $N/100$ hydrochloric acid could be observed, and the constricting elements were visible. When a load was applied, the fibre increased in length and decreased in thickness, the constricting elements becoming less and less apparent. Loads of 7 g. generally brought about the total suppression of the constricting effect and loads of 7 to 10 g. generally caused the fibre-bundles to break. The breaking was similar to that of an ordinary worsted yarn, the individual fibres sliding past each other, the two broken ends being frayed. The width of the different fibre-bundles used in these experiments varied between about 0.10 and 0.30 mm.

Owing to the difficulty of cementing the fibres with paraffin wax, all the later experiments were carried out by holding the fibre-bundles by means of two gold pen nibs. One of the nibs was fixed to a glass slide by means of plasticine and the other was allowed to hang freely, thus putting a constant load on the fibre. The glass slide could then be placed in a rectangular trough containing the experimental liquor and the fibre-bundle viewed through a microscope.

The fibre-bundle was treated with distilled water and its length measured by means of an eyepiece micrometer. This length was divided by five and the width of the fibre-bundle was determined at each fifth of its length. The average width was then calculated and from these two values the approximate volume of the fibre-bundle was determined, assuming that the bundle could be considered truly cylindrical. The fibre was then treated with solutions of hydrochloric acid of decreasing p_H , similar measurements of length and width being taken. The fibre-bundle was passed successively through the various solutions and was then thoroughly washed in distilled water. This same fibre was then treated in the same way and again washed and used a third time. The results are plotted in Fig. 1, which shows the influence of the p_H of the solution on the volume of the fibre-bundle. (The units of volume used in the figures are arbitrary micrometer units.)

The figure shows that the maximum swelling of a fibre-bundle of pure collagen occurs at p_H 1.8–1.9. It will be seen that taking the fibre through the

series of liquors up to p_H 0.3 brings about a change in the fibre-bundle, in that the apparent volume, when it is washed in distilled water after being used the first time, is greater than the original volume. After using twice, however, the apparent volume at p_H 5.0 is less than the original volume. This apparent decrease in volume is due to the breaking down of the fibre-structure, the fibre-bundle having a frayed appearance, and it is difficult to measure with any assured accuracy the exact width of the fibre. Owing to this fraying of the

Fig. 1.

fibrils, it was not possible to use the fibre-bundle more than three times. The increased volume of the fibre at p_H 3.1–5.0, when used the second time, can be interpreted as due to the “opening up” of the fibre-bundle, *i.e.* the loosening of the finer elements of the fibre.

The curves show that increase in swelling only begins at p_H about 3.5 or less. It will be shown that where a marked increase in volume occurs in the range p_H 5.0–3.5, that this increase is not due to “plumping” but to “opening up.” The more a fibre has been used, the more nearly horizontal is the part of the

curve between these p_H values. This means that opening up is a permanent change and that when it is complete the state so brought about is independent of p_H , at any rate (in the case of pure collagen) immediately on the acid side of p_H 5.0 and when hydrochloric acid is used to alter the p_H .

The curves in the region more acid than p_H 1.8 show a point of inflection at about p_H 1.1, when the fibre is used for the second and third times. In some cases this change in slope is found when the fibre is used for the first time, but when this occurs the point of inflection is generally at a slightly higher p_H . A careful examination of the graph shows that there is a slight indication of change of slope in the swelling curve of the fibre when used the first time, the point of inflection being at about p_H 1.6. This break in the curve was noted in the previous work of Kaye and Jordan-Lloyd [1924] and was found to be due to a structural change (splitting or opening up) of the fibre-bundle. It appears possible, however, that this type of opening up is rather different from that which can occur at $p_H > 3.0$. It also appears that the more the fibre-bundle is broken down by continued use, the less sharply defined is the point of maximum swelling. The general characteristic of the fibre-bundles appears to be that they become less and less sensitive to the action of reagents.

In the later work it was decided to abandon the method of direct measurement of the fibre-bundles, owing to its very tedious character, and to take photographs of the fibre-bundles at a known magnification and then to measure the photograph for length and to deduce its area by weighing the carefully cut out picture of the fibre-bundle. From these two measurements, the average width of the fibre-bundle could be computed and the volume calculated on the assumption that a cylinder, of which the length was that of the bundle and the diameter was that of the average width of the fibre-bundle, was equivalent in volume to the fibre-bundle.

The effect of the p_H on the swelling of a fresh hide fibre-bundle is shown in Fig. 2. This curve differs in several details from that shown in Fig. 1. There is a very marked increase in volume in the region p_H 3.5–5.0. This increase is due to the opening up of the bundle. The maximum swelling occurs at about p_H 2.8 and not at p_H 1.9 as in the pure collagen fibres. The significance of this difference will be discussed later.

The point of inflection in the region p_H 1.0 to 2.0 is at about p_H 1.6, whilst there is another break in the curve at about p_H 1.0. The significance of these two points is not yet known.

This method of expressing the results does not, however, throw light on the different ways in which the fibre can hold water. It may be convenient here to note that increase in volume may be brought about by three distinct processes. It can be caused by (a) an osmotic effect, (b) hydration of the protein, by which the water is bound to the protein bringing about an increase in bulk, (c) a physical separation of the component parts of the fibre-bundle. This last type of separation can be brought about by mechanical means, such as constant flexing or by alternately swelling and de-swelling the fibre. It is also possible

that the hydration of the protein leads to a separation of the individual structural units from each other. At the present stage of this investigation, however, it is not possible to distinguish between (b) and (c) and these two effects, which are probably closely inter-related, will be classed under the heading of "opening up." The osmotic uptake of water (a) has quite a different effect, and the difference between it and "opening up" can be clearly shown from the results already obtained.

Fig. 2.

It was mentioned above that increasing the load on a fibre-bundle prevented the violent contortion which is normally produced by the action of $N/100$ hydrochloric acid, and that the constrictions in a swollen fibre caused by the encircling bands of reticular tissue could be made to disappear. Thus a fibre which was stretched could not "plump." This infers, therefore, that "plumping" is characterised by a contraction in length and an increase in thickness. Previous microscopical work has indicated that "opening up" is characterised

by an increase in thickness *only*. There is no apparent contraction in length. If these two dimensions of the fibre-bundle are plotted separately, it would be expected, therefore, that the decrease in length would be paralleled by an increase in width (or thickness) when the fibre-bundle was "plumped" but if "opening up" were taking place as well, the width would increase and would reach its maximum at a point different from that of minimum length. These effects are shown in Figs. 3 and 4, in which the alterations of length and breadth

Fig. 3.

are expressed as percentages of the original dimensions of the fibre-bundle when in equilibrium with distilled water and are plotted against the p_H of the solution.

Fig. 3 shows the behaviour of a fibre-bundle of pure collagen. It will be seen that the first time the fibre is used the length does not begin to decrease appreciably until the p_H of the solution is less than 3.1. The increase in width, however, takes place at a slightly higher value (p_H 3.4). This suggests that some

"opening up" is taking place although its amount is not very great. The remaining portions of the two curves are almost inverse pictures of each other, the position of the maximum width occurring at about the same p_H (1.75) as the minimum length. When the fibre is used a second time, the width is appreciably greater than the original width because of the irreversible structural changes brought about in the first set of liquors. The increase in width with decrease in p_H begins more slowly than when the fibre is first used, but in this

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Fig. 4.

case it is accompanied by a definite, though slight, decrease in length. This suggests that the volume increase is more of the nature of "plumping." When the fibre is used the third time, the width does not increase until about p_H 3.0 is reached and no alteration occurs in the length until the acidity is greater than p_H 2.5, which may be interpreted as an indication of a small amount of "opening up."

Fig. 4 shows the results obtained by using a fresh ox hide fibre-bundle, and, apart from the greater amount of swelling, they differ considerably from those obtained when a fibre-bundle of pure collagen is used.

The increase in width is extremely rapid between p_H 5.0 and 3.5 and it is accompanied by only a comparatively small decrease in length. The decrease in length becomes marked at $p_H < 3.5$ and it is accompanied by a further increase in width. Thus, in this region (p_H 5.0–3.5) there is initially a large amount of “opening up” with a small amount of “plumping,” and it is followed by a region in which “plumping” is probably the dominant factor. This region extends from p_H 3.5 to 2.9. At about p_H 2.8, the width reaches its maximum and there is a change in the rate of decrease in length. (This break in the curve which is similar to that which occurs in the case of pure collagen fibres, takes place, however, at a slightly different point, the p_H being 2.3–2.9 in the case of fresh hide and 1.9–2.5 for pure collagen.) The width of the fibre decreases as the p_H decreases from 2.7 to 2.0, but this decrease is accompanied by a further decrease in length. That is to say, the width is decreasing although the “plumping” is increasing. The decrease in width is caused, therefore, by a decrease in the “opening up” and not by a decrease in “plumping.” The maximum “plumping” occurs at p_H 2.0, a value which is not very dissimilar from that for pure collagen.

The remaining portions of the curves, *i.e.* in the region more acid than p_H 2.0, have certain features similar to those found in the case of pure collagen, especially in the region p_H 0.6–1.2. In addition, there appears to be a break in the curve between p_H 1.2 and 2.0, but until further work has been carried out, it is not possible to interpret the significance of this.

SUMMARY.

A microscopical examination of collagen fibre-bundles has been made using a method whereby the fibre-bundle is held so that accurate measurements can be made. By this technique it is shown that repeated swelling and de-swelling of the fibre-bundle causes it to become less sensitive to the swelling action of dilute acid solutions. It is also shown that the two factors, “plumping” and “opening up” can be distinguished one from the other and can be expressed in a general way by means of graphs. The point of maximum swelling of a fibre-bundle is that point where the sum of the swelling due to “plumping” and that due to “opening up” is greatest. This point is moved to a slightly higher value of p_H in the case of a fibre-bundle from fresh hide, where the amount of “opening up” is great, since the maximum “opening up” probably occurs at about p_H 3.0 to 3.5.

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REFERENCE.

Kaye and Jordan-Lloyd [1924]. *Proc. Roy. Soc. Lond. B* **96**, 293.