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THE MECHANICAL PROPERTIES OF THE UTERINE CERVIX OF THE RAT DURING INVOLUTION AFTER PARTURITION

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We have previously described the change in mechanical properties of the uterine cervix of the rat which enables the foetus to pass through this normally narrow rigid canal at the end of pregnancy (Harkness & Harkness, 1959*b*). It was found that there was an increase in the circumference of the collagenous framework of the cervix, together with a change in its nature, so that it became readily distensible by prolonged small forces. We have now studied the reversal of these changes after parturition and their relation to the rapid removal of collagen from the uterus and cervix which takes place at this time (Harkness & Harkness, 1954; Harkness & Moralee, 1956). Within 24 hr after parturition, the circumference of the collagenous framework decreases to one third of the value at parturition. The property of extensibility under prolonged loading is lost. There is, subsequently, a slower return of the circumference to approximately the non-pregnant value. The alterations in the mechanical properties of the cervix take place more rapidly than loss of collagen. A preliminary account of this work has already been published (Harkness & Harkness, 1958).

METHODS

The animals were albino rats of the local strain. The methods used for examining the excised cervix have previously been described (Harkness & Harkness, 1959*b*; Cullen & Harkness, 1960). It is sufficient to say that two types of measurement were made.

Dimensional. The inner circumference of the cervix at all levels of tension, applied rapidly (minutes) to the point of rupture (from which the *tensile strength* was derived). Symbols: l_z, circumference (mm) at zero tension obtained by linear extrapolation (= a, Harkness & Harkness, 1959*b*); k, the difference between l_z and circumference at breaking tension (= b, Harkness & Harkness, 1959*b*); l_1 circumference (mm) under one standard condition (see Harkness & Harkness, 1959*b*).

Viscous extensibility. The rate of increase in circumference under prolonged tension (hours); the term 'viscous' extensibility is used, since the tissues under test behave like a viscous element in a rheological model, extending at constant rate under a constant load. Symbols: K/l_0 where K is rate of increase in circumference (mm/min) and l_0 is circumference (mm) obtained by linear extrapolation to zero time. K/l_0 is, therefore, the fractional increase in circumference per unit time. The relation of this to tension is not a simple proportionality and has not yet been fully worked out. Comparisons between tissues may be made at known values of tension.

TABLE 1. Composition and mechanical properties of cervix at different times after parturition

	During parturition			4–8 hr			1 day						
	†22°	37°	22° (Pap)	2–4 hr 22°	22°	37°	22° (Pap)	22°	37°	2 days 22°	3 days 22°	4 days 22°	8 days 22°	16 days 22°
1. No. of rats	4	5	4	4	5	4	4	4	2	6	5	6	5	5
2. Body weight at death (g)	217 ±19	239 ±9	268 ±18	199 ±24	199 ±8	192 ±16	205 ±15	191 ±9	188 ±18	198 ±7	188 ±9	226 ±5	224 ±11	255 ±18
3. Weight of cervix (mg)	258 ±28	252 ±26	273 ±12	255 ±4	204 ±10	209 ±34	252 ±14	146 ±16	131 ±3	107 ±5	75 ±7	55 ±2	35 ±2	29 ±2
4. Total collagen in cervix (mg)	9·9 ±1·0	9·5 ±0·7	9·8 ±0·3	9·6 ±0·8	8·9 ±0·7	8·5 ±0·8	9·7 ±0·2	8·7 ±0·4	8·6 ±0·6	7·4 ±0·4	4·8 ±0·6	4·2 ±0·4	2·1 ±0·1	2·0 ±0·2
5. Concn. of collagen (g/100 g)	3·9 ±0·1	3·8 ±0·2	3·6 ±0·2	3·8 ±0·3	4·3 ±0·2	4·4 ±0·7	3·9 ±0·2	6·1 ±0·3	6·6 ±0·6	7·0 ±0·2	6·5 ±0·2	7·5 ±0·7	6·7 ±0·2	9·0 ±0·5
6. l_1 (mm)	35·5 ±1·6	46·7 ±4·4	35·4 ±0·6	24·7 ±1·1	9·3 ±1·1	10·2 ±0·8	12·5 ±1·4	9·1 ±0·3	10·9 ±0·1	8·9 ±0·3	7·5 ±0·2	6·5 ±0·3	4·7 ±0·1	4·1 ±0·1
7. l_z (mm)	43·7 ±2·3	*67·8 ±9·8	50·0 ±3·2	35·9 ±0·7	12·2 ±2·2	8·9 ±2·2	14·9 ±2·9	11·7 ±0·6	12·2 ±0·3	11·6 ±0·7	9·7 ±0·4	8·2 ±0·3	5·2 ±0·1	4·7 ±0·1
8. k/l_z	0·29 ±0·03	*0·20 ±0·03	0·22 ±0·02	0·37 ±0·05	0·48 ±0·04	0·51 ±0·17	0·51 ±0·04	0·37 ±0·02	0·44 ±0·01	0·41 ±0·04	0·38 ±0·02	0·37 ±0·03	0·33 ±0·05	0·30 ±0·06
9. Break circumference (mm)	56·8 ±2·9	79·3 ±7·0	61·3 ±4·4	51·2 ±2·9	19·4 ±3·2	19·4 ±2·8	23·3 ±4·1	16·2 ±0·6	17·8 ±0·4	16·5 ±0·9	13·5 ±0·4	11·4 ±0·6	7·5 ±0·5	6·2 ±0·4
10. Break tension (kg/mm² collagen)	1·13 ±0·12	*0·68 ±0·14	1·19 ±0·10	1·28 ±0·09	0·87 ±0·17	0·74 ±0·13	1·01 ±0·21	1·41 ±0·10	0·86 ±0·10	0·99 ±0·09	1·13 ±0·08	1·09 ±0·14	0·84 ±0·11	0·84 ±0·09

The estimate of variation is the standard error of the mean. The meanings of l_1, l_z and k are given in the text.

† Temperature of test.

* Based on 3 animals in which cervix did not rupture under initial 50 g loading. (Pap) = tests done in presence of papaverine sulphate 50 mg/l.

Chemical

All samples were placed in 5 ml. 6N-HCl at the end of tests and hydrolysed for 4 hr at 40 lb./sq.in. (2·8 kg/cm²) pressure in an autoclave. The hydroxyproline content of the hydrolysate was estimated by the method of Neuman & Logan (1950), and the collagen content of the sample estimated by multiplying this by 7·46.

RESULTS

Circumference of cervix. The results of experiments concerned with the changes in dimensions of the cervix are recorded in Table 1 and Figs. 1 and 2. Circumference (line 7, Table 1) falls in 8 hr to about one third of that at parturition. Subsequently there is a further, slower fall, until by the eighth day the value is about an eighth of that at parturition. There is also a fall in the weight of cervix (line 3), accompanied by loss of collagen (line 4), these changes becoming evident 24 hr after parturition. The latter change proceeds more slowly so that there is a rise in concentration of collagen.

Tensile strength of tissue. The absolute load required to break the tissues rose after parturition and then fell (Fig. 3). The explanation appears to be that at first the walls of the cervix become thicker, because the fall in circumference proceeds faster than loss of tissue, and the breaking load rises. Later there is a loss of tissue, and the walls become thinner and hence weaker. Breaking tension per unit cross-sectional area of collagen shows comparatively little variation over the whole period of study (Table 1, line 10). The figures in Table 1 are calculated from inner circumference. If mean circumference is estimated (mean between inner and outer) and used instead, the correction at the early stages after parturition is small (about 5%) but rises to about 30% at 16 days. The effect is to reduce the differences in tensile strength per unit cross-sectional area of collagen between groups.

Extensibility. Measurements were made at only one time (24 hr) after parturition. Results are given in Table 2. Values of K/l_0 for the comparable tension per unit cross-sectional area of collagen 24 hr after parturition are similar to those found in the normal cervix, and 30–40 times lower than at parturition (Harkness & Harkness, 1959*b*).

TABLE 2. Extensibility of cervix 24 hr after parturition

l_1 (mm)	16·8 ± 1·7
l_0 (mm)	18·1 ± 1·8
$10^3\ K/l_0$	0·59 ± 0·18
Tension at l_0 (g/mm² collagen)	490 ± 77
Tension at break (kg/mm² collagen)	1·55 ± 0·19

The estimate of variation is the standard error of the mean.

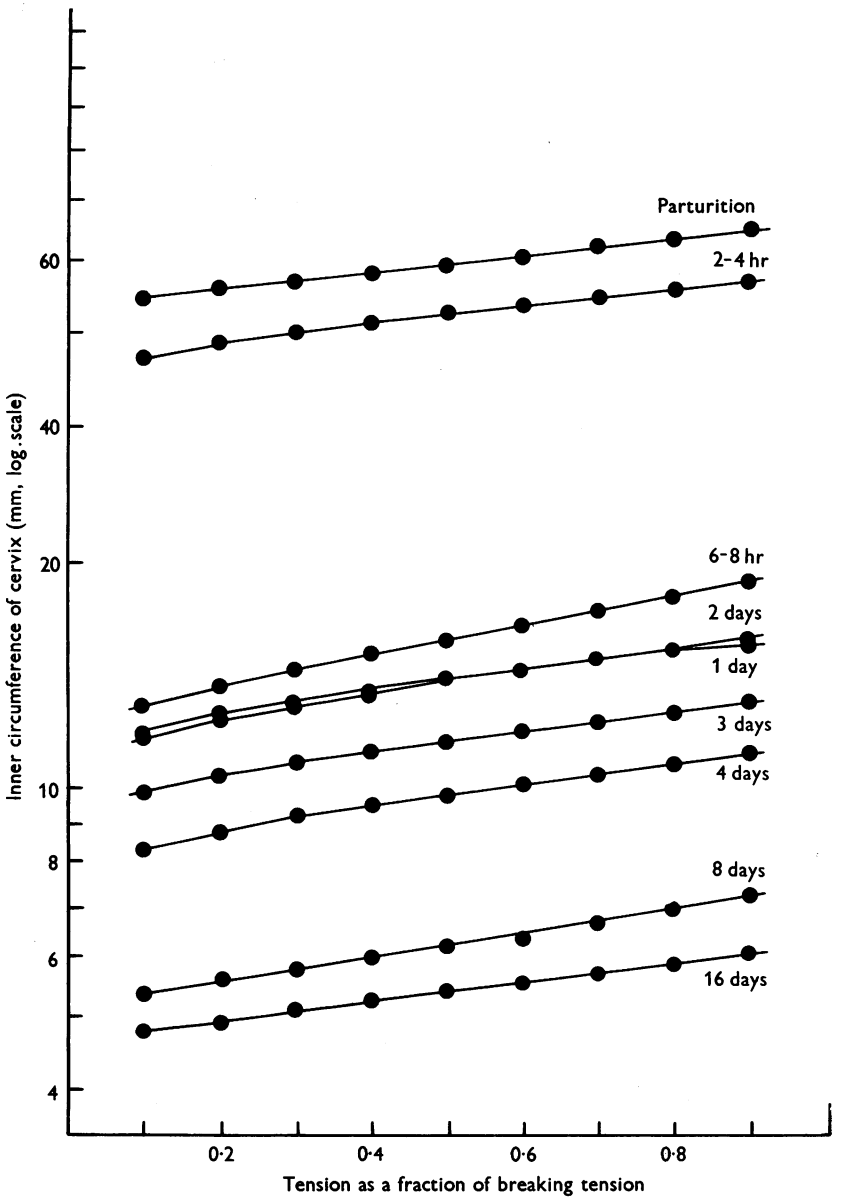

Fig. 1. The relation of inner circumference of cervix to tension. The curves represent mean values for all animals in each group. The time after parturition is given against each curve. Tension (abscissa) is given as a fraction of breaking tension. Samples tested at 22° C. Semi-log. scale.

DISCUSSION

Comparison with changes in pregnancy

It is of interest first to consider the relation of our results to those previously found in pregnancy (Harkness & Harkness, 1959*b*). The circumference of the cervix during parturition is rather more than twice as great as on the last day of pregnancy before parturition (21st day), and this in its turn is 3–4 times as great as in the non-pregnant animal and up to the

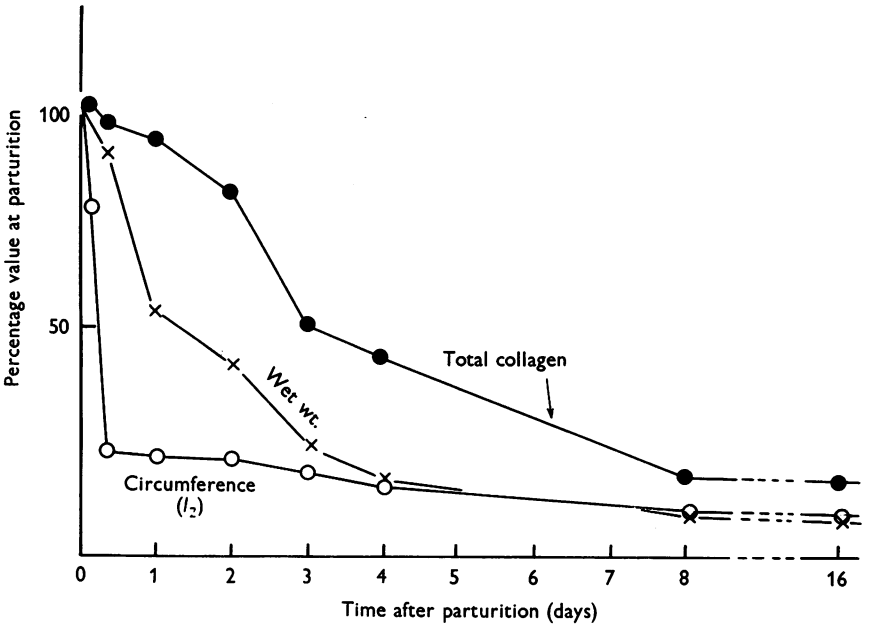

Fig. 2. Comparison of time courses of change in total collagen, wet weight and inner circumference of cervix after parturition. The values of wet weight and total collagen are means for all rats. The measure of circumference is l_z, for samples tested at 22° C. All values are given as percentage of the value at the time of parturition.

12th day of pregnancy. Thus, it is evident that there is, comparatively, a very large change in the last 24 hr or so before parturition. After parturition the course of change in circumference is more rapid than that which precedes it.

For comparison with measurements of the circumference of the cervix made during parturition it may be stated that the circumference of the foetal head is about 35 mm (Harkness & Harkness, 1959*b*). This is rather less than the value of l_z, obtained at parturition by extrapolation of the length–tension curve to zero load, which may be taken as a measure of the size of the unstretched collagenous framework. The reason l_z is not equal

to the size of the foetal head, but slightly larger, is probably because there is a thin partition across the upper part at least of the cervical canal, dividing the two sides so that an individual foetus does not have unhindered use of the whole circumference we have measured, but dilates the cervix asymmetrically. This explanation is borne out by a single instance that we observed of a rat, killed during parturition, which proved to have foetuses in only one horn. In this animal dilatation of the cervix was asymmetrical.

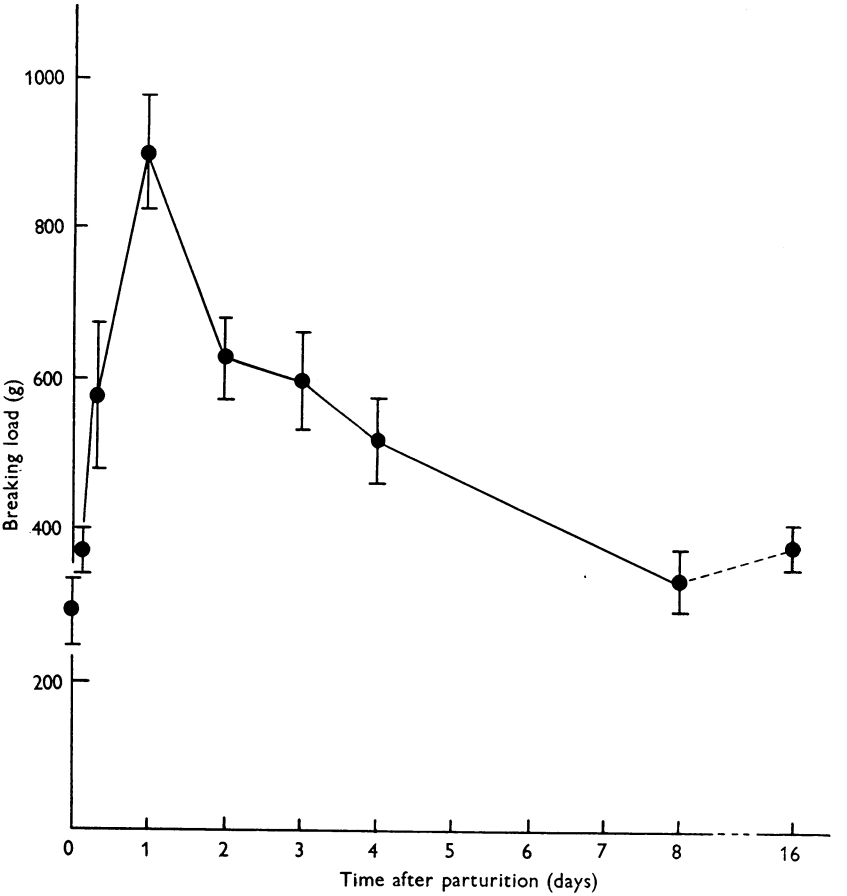

Fig. 3. Breaking load at different times after parturition. The length of the vertical line through each point is twice the standard error of the mean. Tests done at 22° C.

Changes in the first day after parturition

The changes after parturition fall roughly into two phases, an early one of rapid change and consolidation of the tissue in the first day, and a later one of slow change associated with the general involution of the reproductive tract which occurs *post partum* after the first 24 hr. In the first

phase, immediately after parturition, a reduction in circumference is presumably brought about by contraction of smooth muscle. This, however, is rapidly made permanent by a consolidation of the tissue; the property which we have called viscous extensibility is lost, and the tissues cannot be stretched again. It is obvious from the nature of this consolidation that it cannot be the result of muscular activity, but must reflect a change in the collagenous framework; it takes place with little or no alteration in the total collagen of the tissue. This fact, and the rapidity of the change, make it unlikely that there is removal of collagen and replacement by a newly formed framework of smaller size. It seems probable that the property which we have called 'viscous extensibility' involves slip between the collagen fibrils or fibres. The unit of collagen structure in tissues is well known to be the fibril, a cylindrical structure of unknown but great length, having, in a given tissue, a relatively uniform diameter of usually 500–1000 Å, and consisting, apparently, only of the protein collagen itself. Electron microscopical examination shows these fibrils to be separated by spaces which must, in life, be filled by some material not yet identified. Since collagenous tissue in general appears to show this extensibility only to a small extent, it can be concluded that the fibrils themselves are inextensible. Thus, there are *a priori* grounds for supposing that this type of extensibility, when found, involves slip between, rather than within, the collagen fibrils. The fact that extensibility is greatly increased by the proteolytic enzymes trypsin and chymotrypsin (Harkness & Harkness, 1959*a*), which have been found to have little or no apparent effect on collagen when in solid form, has to be used to support this hypothesis. However, in view of the recent finding of Hodge, Highberger, Deffner & Schmitt (1960) that trypsin can attack the free ends of the collagen molecule in solution, this argument is of doubtful validity, and the nature of the slow extension remains obscure.

It is customary to regard the collagenous framework of connective tissue as a relatively permanent, fixed structure, and in general it is clear that this view is correct. The tissues of the genital tract have previously been found to be exceptional in that they can destroy collagen after parturition at such a rate that the time to half loss is only 1–2 days in the rat, considerably less than the half life, in this animal, of such metabolically active material as plasma protein. It is now clear that still more rapid changes can take place in the mechanical properties of the collagenous framework of the genital tract, but it seems unlikely that the physiological importance of the properties we have been investigating is confined to this tract. One might expect, for example, that similar changes are involved in the closure of the ductus arteriosus after birth.

Comparison of later changes with contraction in wounds

In the second phase of the changes in the cervix, from 24 hr on, there is a much slower fall in circumference which roughly parallels the general involution of the tissue, shown by fall in weight and total collagen content. Change in circumference, which is a linear dimension, cannot be compared directly with change in total collagen content, but might be expected, if the two were linked, to be proportional to the cube root of the latter. Although the relation does not hold exactly, the circumference falling rather more than would be expected, it is nevertheless clear that the two changes proceed with similar time courses.

The later change in circumference of the cervix resembles the 'contraction' of wound tissue, and it is reasonable to suppose that similar processes in the connective tissues are involved in both. The process of wound contraction in skin, where it has principally been studied, consists in the drawing together of the edges of the wound and reduction in the area enclosed by them. Both processes diminish in rate with time, and can be described, approximately, in terms of one or more exponential time constants. From 24 hr to the eighth day the contraction of the circumference of the cervix has a half time of 5–6 days. Times of half reduction of linear dimensions for wounds, assumed proportional to square root of area, vary both with time after wounding and between experiments, from as low as 2–4 days (Van den Brenk, 1956; Grillo, Watts & Gross, 1958*a*, *b*) during the most rapid phase of contraction, to 2 months or so in the later phases (Abercrombie, Flint & James, 1954, 1956; Billingham & Medawar, 1955; Billingham & Russell, 1956). Although greater changes have been reported in wounds, in the majority of reports the extent of contraction is similar to that found in cervix, i.e. to between $\frac{1}{2}$ and $\frac{1}{3}$ the original linear dimensions. Quantitatively the two processes are therefore comparable. Another resemblance is that in both, during the contraction, there is an absolute loss of substance from contracting tissue and the material lost includes collagen (Grillo *et al.* 1958*a*, *b*).

The nature of wound contraction and the origin of the forces which bring it about are still disputed (see Abercrombie, James & Newcombe, 1960). Experiments such as those reported here have not been done on wounds, but it is reasonable to suppose that in them also the contraction involves a diminution in the size of the collagenous framework. None of the hypotheses so far put forward has taken this into consideration.

SUMMARY

1. Changes in the circumference and mechanical properties of the cervix uteri of the rat, from the time of parturition to 16 days after, have

been investigated by stretching the tissue between two parallel rods, one through each canal.

2. There was a rapid decrease within 8 hr in the circumference of the collagenous framework of the cervix, to about one third the value at parturition, followed by a slower reduction to a third again in the next 2 weeks to a value near the normal.

3. The initial decrease in circumference takes place with little change in the total collagen content of the tissue, but the later change is associated with the general involution of the tissue which occurs after parturition.

4. Within 24 hr after parturition the collagenous framework of the cervix loses the ability which it develops during pregnancy, to extend continuously under prolonged low loads, and its behaviour resembles that of a normal non-pregnant cervix.

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