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Cervical tissue shrinkage by formaldehyde fixation, paraffin wax embedding, section cutting and mounting

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Summary. To evaluate the efficacy of cryocoagulation as a treatment for cervical intraepithelial neoplasia (C.I.N.), it is necessary to know the maximum depth of the glandular crypts, the maximum crypt involvement by C.I.N. and the extension of the cryolesion, obtained under standardized conditions. In a morphometric study on this subject, one has to take into account the shrinkage of the cervical tissue, caused by processing the tissue for histological examination.

In the present study, tissue shrinkage of the cervix in different directions was measured in three separate steps. First shrinkage caused by formalin fixation was determined, second shrinkage caused by dehydration, clearing and paraffin wax embedding and finally that caused by section cutting and mounting.

Shrinkage caused by formalin fixation, and by dehydration, clearing and paraffin wax embedding did not differ significantly in the different directions and resulted in an average shrinkage of respectively 2.7% and 12.6% of the original dimensions. The alterations of the dimensions by section cutting and mounting is not a process of shrinkage, but actually a deformation caused by pressure on the tissue during sectioning. Generally the dimension decreases in the cutting direction and increases in the direction perpendicular to it. In the calculation of the total shrinkage these alterations can be neglected, since the changes, although not consistent are small.

It follows that in morphometric studies a total shrinkage of about 15% of the original dimensions has to be taken into consideration.

Key-words: Cervix uteri – Shrinkage – Histological technics – Formaldehyde – Paraffin – Sectioning-mounting

With the reintroduction of the colposcope as an aid in the diagnosis of cervical intraepithelial neoplasia (C.I.N.) the various methods for local treat-

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ment of these lesions have become more and more important. When using tissue destructive methods, it is necessary that the destruction is extensive enough to eradicate all the pathological cells, particularly those located in the endocervical canal or in the glandular crypts. In the case of cryotherapy, there is a lack of adequate data as to the effective depth of tissue destruction (Charles and Savage 1980; Singer and Walker 1982). To guarantee that destruction by cryocoagulation is deep enough to embrace the lesions in their entirety, one ought to know the maximum depth of the glandular crypts, the maximum crypt involvement by C.I.N. and the 3-dimensional extension of the cryolesion obtained under standardized conditions. Before using cryosurgery as a treatment for C.I.N., we decided to investigate these problems morphologically.

The processing of tissue for morphological investigation results in shrinkage, which should be taken into consideration when slides are compared with living tissue, for example in histometric studies (Hopwood 1982). For this reason we decided to investigate the amount of shrinkage caused by tissue processing.

Material

The cervices of patients who underwent a hysterectomy for benign disease were used for this study. In all cases a benign condition of the cervix was verified by preoperative cytological screening and postoperative histological examination.

Methods

After hysterectomy the cervices were amputated from the unfixed operation specimens.

The shrinkage caused by the subsequent steps in tissue processing, was measured in several different ways and directions. First the shrinkage caused by fixation was measured, then that caused by dehydration, clearing and paraffin wax embedding and finally that caused by section cutting and mounting.

Shrinkage caused by fixation

The dimensions of the fresh and fixed specimens were compared. We measured the following four dimensions of the amputated specimens before and after fixation for 24 h in 8% formalin (see Fig. 1).

1. The length of the amputated part of the cervix (the longitudinal diameter = A).
2. The antero-posterior diameter of the portio (the distal antero-posterior diameter = B).
3. The transverse diameter of the portio, perpendicular to B (the transverse diameter = C).
4. The antero-posterior diameter at the level of amputation (the proximal antero-posterior diameter = D).

In this way 25 cervices were examined and the results before and after fixation were compared to give the percentage of shrinkage by 8% formalin in these different directions. Other types of fixative, such as Methacarn (methanol carnoy mixture), Bouin's fluid (picric acid), sulphosalicylic acid and mercuric chloride solution have also been tested.

Shrinkage caused by dehydration, clearing and paraffin wax embedding

The size of the tissue blocks before and after embedding was compared. For this purpose 25 amputated formalin fixed cervices were sectioned in two ways: (see Fig. 2)

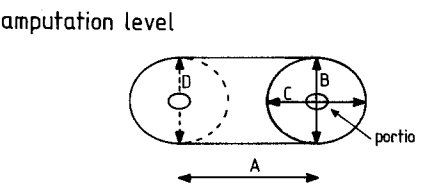

Fig. 1. Schematic representation of the measurement directions: longitudinal (*A*), distal antero-posterior (*B*), transverse (*C*) and proximal antero-posterior (*D*) diameter of the amputated cervix uteri

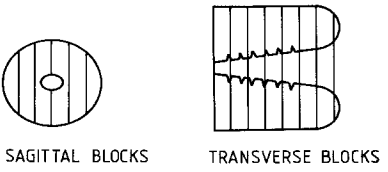

Fig. 2. The section direction of the fixed amputated cervix in sagittal and transverse blocks

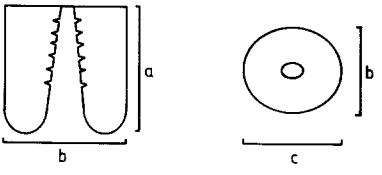

Fig. 3. Schematic representation of the measurement directions: longitudinal (*a*), antero-posterior (*b*) and transverse (*c*) diameters of the sagittal and transverse blocks

1. In sagittal blocks perpendicular to the external os (in the antero-posterior planes) with a thickness of 2.5 mm.

2. In transverse blocks with a thickness of 2.5 mm each.

Life-size photographs of all the slices were made and the greatest diameters were measured in two directions, namely: in the sagittally cut blocks in the longitudinal and antero-posterior directions, respectively a and b in Fig. 3a; and in the transversely cut blocks in the antero-posterior and transverse directions, respectively called b and c (see Fig. 3b).

Next, the processes of dehydration, clearing and embedding of the blocks were performed automatically in a histokinette.

Dehydration was carried out with alcohol of increasing strength, beginning with 70% alcohol, then 96% and finally with absolute alcohol. Xylene was used as clearing agent. Xylene fulfils the essential requirement that a clearing agent is miscible with both the dehydration agent and the embedding agent (Gordon 1982). Because of the possible carcinogenic properties of methylbenzoate and benzol, recommanded by Burghardt (1972) we prefer to use xylene.

Finally the tissues were embedded in paraffin and blocked out, using the so-called Leuckhardt's 'L' pieces (Gordon 1982). From each tissue block embedded in paraffin the above described dimensions (a, b, c) were measured and compared with those obtained from the photographs. In this way the percentage shrinkage caused by dehydration, clearing and embedding could be determined.

Shrinkage caused by section cutting and mounting

The size of embedded tissue blocks and sections of them were compared. In tissue blocks prepared as described, in longitudinal, antero-posterior and transverse direction, three pairs of recognizable points, "landmarks", on the circumference of the tissue blocks were pinpointed, allowing measurement in these three directions. After this the sagittal blocks were sectioned in longitudinal direction (from proximal to distal) and the transverse blocks in antero-posterior direction. After cutting and mounting the "landmarks" could be traced in the slides. Again the distances were measured and the differences with the measurements in the blocks recorded.

Results

Shrinkage by fixation

In 25 amputated cervices, the measurements described above were made. The median shrinkage by 8% formalin fixation, in both, distal and proximal antero-posterior diameter (B and D, Fig. 1) was found to be zero.

In the longitudinal direction (A, Fig. 1) the median shrinkage was 3.0%, and in the transverse direction (C, Fig. 1) it was 2.4% (see Table 1).

Shrinkage by dehydration, clearing and paraffin wax embedding

Sagittal blocks. To evaluate the influence of columnar epithelium upon the shrinkage, measurements were performed separately in two different groups of sagittal blocks. One group consisted of 51 sagittal blocks, without any columnar epithelium due to the fact that the cutting level was outside the endocervical canal. The other group was composed of 78 sagittal blocks, which were cut through the endocervical canal and, therefore, included columnar epithelium. In each group the percentage of shrinkage was determined in the two directions a and b (longitudinal and antero-posterior directions, Fig. 3).

In the first group the mean shrinkage for a was $12.8 \pm 1.2\%$ and for b $13.9 \pm 2.4\%$ of the fixed specimen dimensions. In the second group $11.0 \pm 1.3\%$ and $15.1 \pm 1.3\%$, respectively (see Table 2).

Transverse blocks. In 113 transverse blocks the shrinkage was measured both in the antero-posterior and transverse directions (b and c respectively in Fig. 3). For b the mean shrinkage proved to be $13.9 \pm 2.4\%$, for c it turned out to be $12.6 \pm 2.8\%$ (see Table 2).

Shrinkage by section cutting and mounting

Two hundred and sixty determinations have been performed in sagittal paraffin blocks. In longitudinal direction (=a, see Fig. 3), the cutting direction, there was a mean decrease of the distance between the two points of $2.2 \pm 1.8\%$. In antero-posterior direction (=b, see Fig. 3), perpendicular on the cutting direction a mean increase of $0.4 \pm 1.2\%$. In transverse blocks

Table 1. Cervical shrinkage by 8% formalin fixation

Median shrinkage and range in different directions in percent of original dimensions			
Longitudinal	Distal antero-posterior	Transverse	Proximal antero-posterior
A	B	C	D
3.0% (0–7.5) $N=25$	0% (0–3.6) $N=25$	2.4% (0–5.3) $N=25$	0% (0–6.7) $N=25$

N = Number of examined specimens

Table 2. Cervical shrinkage by dehydration, clearing and paraffin wax embedding

	Mean shrinkage + standard deviation in different directions in percent of the fixed specimen dimensions					
	Longitudinal a	*N*	Antero-posterior b	*N*	Transverse c	*N*
Sagittal blocks						
without columnar epith.	12.8±1.2%	51	13.9±2.4%	51		
with columnar epith.	11.0±1.3%	78	15.1±1.3%	78		
Transverse blocks			13.9±2.4%	113	12.6±2.8%	113
Mean value	11.9%	129	14.3%	242	12.6%	113

N = Number of determinations

Table 3. Cervical tissue deformation caused by sectioning, cutting and mounting

	Mean decrease (+) and increase (−) + standard deviation in different directions in percent of the paraffin wax embedded tissue dimensions					
	Longitudinal a	*N*	Antero-posterior b	*N*	Transverse c	*N*
Sagittal blocks	+2.2±1.8%	260	−0.4±1.2%	260		
Transverse blocks			+4.3±2.2%	32	−3.0±1.5%	32

N = Number of determinations

32 measurements have been made. This resulted in a mean decrease of 4.3±2.2% in antero-posterior direction (=b, see Fig. 3), the cutting direction, and a mean increase of 3.0±1.5% in transverse direction (=c, see Fig. 3), perpendicular on the cutting direction (see Table 3).

Discussion

From the literature little information is available about the shrinkage of tissues, especially cervical tissue, caused by fixation, processing, embedding, cutting and mounting. Berg (1908) measured the volume of a liver before and after fixation in formaldehyde 4% aq., dehydration with absolute ethanol and embedding in melted paraffin wax. The mean volume after this procedure was 68% of the volume of the fresh organ, that means a shrinkage of 32%. The amount of shrinkage due to fixation alone was only 1%. Przybora (1959) performing a histological study of the topography of carcinoma in situ of the uterine cervix, calculated this shrinkage coefficient by measuring the length of the cervical canal in 15 excised cervices before and after fixation in 10% formalin and paraffin wax embedding. He found a shrinkage of about 20%.

More recently, Anderson (1980), who investigated the depth of the crypts and the depth of crypt involvement by C.I.N., stated that the shrinkage was less than 5% and could be neglected. It is remarkable that he used Bouin's fixation fluid, which contains picric acid, an agent which is generally well known to cause considerable shrinkage of tissue (Hopwood 1982; Berg 1908; Baker 1960). Puchtler et al. (1970) found that Methacarn caused little or no shrinkage. Our experience is that it is difficult to section Methacarn fixed specimens, because of wrinkling of the tissue. This effect may be the result of exposure of tissue to water which is known to cause disastrous effects, such as severe shrinkage artifacts (Puchtler et al. 1970). Also other fixatives tested (Bouin's fluid, sulphosalicylic acid and mercuric chloride solution) caused difficulties in section cutting. As described in the literature (Hopwood 1982) mercuric chloride solution caused excessive shrinkage. Four percent formaldehyde in saline, which is equivalent to 8% formalin, gave the best results.

Specimens fixed for 24 h in this solution suffered less shrinkage and had a perfect consistency for good sectioning. After 24 h most of the formalin could be washed out. Prolonged fixation in formaldehyde, however, causes more shrinkage and hardening of tissue (Hopwood 1982).

In our series we did not find an essential difference in the shrinkage caused by formalin fixation in longitudinal and transverse directions (3.0% and 2.4% respectively, see Table 1). It is interesting that the shrinkage by formalin fixation in the antero-posterior direction, both in distal and proximal parts of the cervix was nil, whilst there is undoubtedly shrinkage of the whole cervix. This might be explained by the fact that the shrinkage is associated with a change in shape of the cervix from oval to round so that the resulting increase in antero-posterior diameter cancels out the real decrease produced by shrinkage.

In longitudinal direction, the shrinkage produced by processing and paraffin embedding was 1.8% more in cervical tissue without columnar epithelium as compared to tissue with columnar epithelium. In antero-posterior direction it was 1.2% more in tissue with columnar epithelium (see Table 2). These differences, although statistically significant, were too small to take into consideration in our morphometric study. Moreover the differences are not consistent in both directions. We concluded that the presence of columnar epithelium does not essentially influence the amount of shrinkage. It follows that the mean antero-posterior shrinkage in sagitally cut blocks is 14.5%. In transversely cut blocks it amounts to 13.9%. The difference of 0.6%, although also significant, is small and neglectable for practical purpose. This implies that the various methods we used, by making measurements of sagittal and transverse blocks, have not altered the results essentially. Based on these observations we feel justified in pooling these results of both groups. Thus the mean shrinkage caused by dehydration, clearing and embedding in the longitudinal direction is 11.9% in 129 determinations, in the antero-posterior direction is 14.3% ($N=242$) and in transverse direction is 12.6% ($N=113$). Based on the observations that the shrinkage, caused by formalin fixation and paraffin embedding, is small in the various directions, it seems permissible for all practical purposes to use the mean

of the values in the different directions, which results in the following figures:
– the overall mean shrinkage caused by formalin fixation is 2.7% of the fresh specimens.
– the overall mean shrinkage caused by dehydration, clearing and paraffin embedding is 12.9% of the fixed specimen, that is $\frac{97.3}{100} \times 12.9\% = 12.6\%$ of the fresh specimen.
– fixation and processing together account for 15.3%.

From 260 determinations in sagittal blocks it was evident that the dimensional alterations of the cervical tissue caused by sectioning and mounting was variable, sometimes an increase and sometimes a decrease in size was found. The decrease in longitudinal dimension corresponded to the cutting direction and the increase in the antero-posterior dimension was perpendicular to this. From this it can be concluded that the alterations are not a process of shrinkage, but actually a deformation of the tissue, caused by the pressure on the tissue during sectioning. To prove this statement we did another 32 determinations in transverse blocks with an antero-posterior cutting direction, the opposite of the procedure in sagittal blocks. Now it was striking that there was a decrease in antero-posterior direction (see Table 3). In sagittal blocks, which will be relied on most in the morphometric analyses, the increase and the concomitant decrease were small and moreover, variable, depending on the cutting direction and pressure. Therefore it is reasonable to neglect the influence of sectioning and mounting in the calculation of the total shrinkage percentage of cervical tissue.

We can conclude from our study that if cervical tissue for morphometric studies has been prepared by formalin fixation, dehydration, clearing, paraffin wax embedding, sectioning and mounting, one has to take into account a total shrinkage of about 15% of the fresh specimen.

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