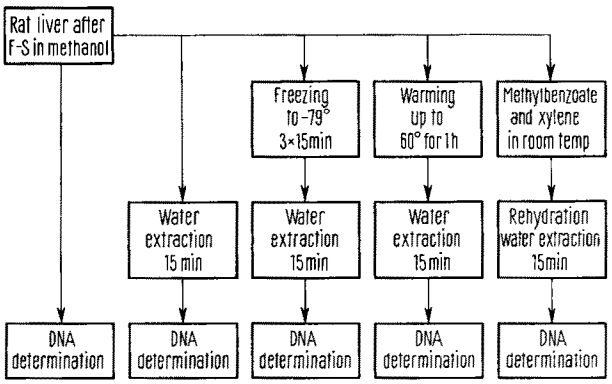
The Possible Losses of DNA from 'Freeze-Substituted' Tissue in the Different Histological Procedures

It was found in a previous paper[1] that 24% of DNA from freeze-substituted rat liver may be lost during histological procedure. There is a lot of evidence that DNA in solution is sensitive to changes of temperature[2-6]. The changes may consist of significant degradation of the macromolecules of DNA. In the course of histological procedure, the tissues are first frozen in freeze substituted procedure and then warmed up to ca. 60 °C for embedding. In this paper the influence of freezing and warming up on DNA content in rat liver was studied, as well as influence of methyl benzoate and xylene as media necessarily used in the histological technique.

Material and methods. The liver of the male rats weighing 150–200 g were used in the experiment. The samples weighing 30–100 mg were cut off from the same liver lobe. Controls were fixed in methanol for 3 h in room temperature, then sectioned on the freezing microtome for 50 μ thick sections. DNA was determined in the untouched sections and in sections extracted by distilled water in room temperature. Experimental fragments were freeze-substituted in methanol in — 79 °C, 12 days long. Sections were prepared afterwards in the same way as the control material. After sectioning, the material was treated in different ways according to scheme in the Figure. Methods of DNA determination, as well as method of statistical analysis, were described previously[1].

Results. In the Table mean values of DNA content expressed as P mg % of fresh weight of tissue are given. In 2 experimental groups significant decrease of DNA content was found: in the freeze-substituted material warmed up to 60 °C and in the freeze-substituted material extracted in methyl benzoate and xylene. Both groups were in the last step extracted by water. The mean results of these groups compared to the control one show the statistically significant ($P < 0.01$) differences. The decrease of DNA content in the material warmed up to 60 °C is 11.4%, and in the extracted by organic solvents material it is 16.3%. In the other groups the differences in comparison with control are small and insignificant.



Experimental groups	No. of repeated experiments	Mean values of DNA expressed as P mg % Mean	SD	SE	DNA (%)
Control	33	16.6	2.6	0.5	100.0
Control, H_2O extract	16	15.6	2.6	0.7	94.0
F-S	17	16.7	3.7	0.9	100.6
F-S, H_2O extract	19	16.4	3.3	0.8	98.8
F-S, 3 times cooled to — 79 °C, H_2O extract	11	16.2	2.9	0.9	97.6
F-S, warmed up to 60 °C H_2O extract	16	14.7	1.7	0.4	88.6
F-S, organic solvents extract and H_2O extract	16	13.9	1.1	0.3	83.7

Discussion. The sum of losses in the material warmed up to 60 °C and material extracted by the organic solvents used is 27.7%. A similar effect: 24% loss of DNA was observed after full histological procedure in F-S tissues[1]. It seems that both warming up and extraction in organic solvents are the main factors in lowering the DNA content in tissues in the course of histological procedure. It is interesting that DNA, which is bound to the structural proteins of nuclei, is sensitive to the increase of temperature. The influence of low temperatures was insignificant, although performed several times on the sample. The losses of DNA from the tissues after procedures described in this paper are dependent on the rise of temperatures and on the extraction of lipids by organic solvents. Extraction of lipids from the sections may increase the water permeability into the cell nuclei. No observation concerning the DNA solubility in organic solvent is known. We have ignored in this paper the discussion of the influence of paraffin wax used for embedding on the possible DNA loss. But it seems not to be an important factor for this problem.

Conclusions. (1) The losses of DNA from the 'freeze-substituted' tissues depend mainly on the 2 stages of histological procedure: warming up to 60 °C and extraction in methyl benzoate and xylene followed by rehydration. The loss of 11.4% and 16.3% of DNA respectively was found in these 2 procedures. (2) Freezing of tissues to — 79 °C, even when performed several times, did not cause significant loss of DNA.

Résumé. Nous avons étudié l'influence de la réfrigération et du chauffage sur le contenu en ADN dans le foie du rat, de même que l'influence des solvents organiques employés dans les techniques histologiques. Les pertes de l'ADN des tissus soumis au «freeze-substitution» dépendent du chauffage à 60 °C (perte 11,4%), et de l'extraction par benzoate du méthyl et xylène suivie de réhydratation (perte 16,3%). La réfrigération ne cause pas de perte importante d'ADN.

J. Komender, H. Kościanek-Malczewska and K. Ostrowski

Department of Histology and Embryology, Medical Academy, Warszawa (Poland), 19 February 1968.

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