VIA MEDICA

TECHNICAL NOTE

Vol. 62, No. 2, pp. 157–161

ISSN 0015–5659
www.fm.viamedica.pl

Application of acrylic emulsion Liquitex R (Binney and Smith) for the preparation of injection specimens and immunohistochemical studies — an observation

Jerzy A. Walocha[1], Wojciech Szczepański[2], Adam J. Miodoński[3], Janusz Gorczyca[1], Janusz Skrzat[1], Tomasz Bereza[1], Piotr Ceranowicz[4], Jacek Lorkowski[1], Jerzy Stachura[2]

[1]*Chair of Anatomy, Medical College of the Jagiellonian University, Kraków, Poland*
[2]*Chair of Pathology, Medical College of the Jagiellonian University, Kraków, Poland*
[3]*SEM Laboratory, Clinic of Otolaryngology, Medical College of the Jagiellonian University, Kraków, Poland*
[4]*Chair of Physiology, Medical College of the Jagiellonian University, Kraków, Poland*

[Received 8 January 2003; Revised 20 February 2003; Accepted 9 March 2003]

Application of acrylic emulsion Liquitex R for injection studies of the vascular system of human myomatous uteri was analysed. It was found that this injection mass does not penetrate the capillary bed of human organs, but it is useful in studies carried out on the blood supply of the human organs removed from cadavers. The results were compared with the studies performed with the help of immunohistochemical tests for von Willebrandt's factor.

key words: acrylic emulsion, Liquitex R, injection, immunohistochemical tests, von Willebrandt's factor

INTRODUCTION

Classical injection technique is contributive in the development of studies of the vascular system of regular anatomical structures, as well as pathological changes, i.e. neoplasmatic tumours [4, 14, 17].

MATERIAL AND METHODS

Acrylic emulsion Liquitex R (Binney and Smith Company, USA) is a professional quality medium, polymer-based paint, which is available in different viscosities. It can be mixed to change paint consistency, drying rate, absorption rate and texture. The emulsion is composed of minute solid particles of plastic resin suspended in water. It dries rapidly, is water insoluble, is highly flexible and does not yellow with age.

The studies were carried out on 30 uteri, obtained from cadavers. The autopsies were carried out in the Forensic Medicine Institute of the Medical College of the Jagiellonian University. The uteri were injected with water solution of the acrylic emulsion (Liquitex R, Binney and Smith, USA). Next the uteri were perfused with heparinised saline (12.5 IU/ml, 37°C), containing 3% Dextrane (molecular weight 70,000) and 0.025% Lidocaine, through ball-needles mounted bilaterally in the uterine vessels, to remove the blood and clots from the vascular bed. The perfusion was continued until the fluid flowing from the vessels was clear and water-like. Next the vascular bed was filled with injection mass, using a 20 ml syringe. The injection was continued until filling of the smallest vessels of the perimetrium, seen with a naked eye. The injected uteri were stained in 10% formaldehyde solution for about two weeks. Next the uteri were cut into slides (6 mm wide). The prepared material was dehydrated in rising concentrations of ethyl alcohol beginning with a concentra-

Address for correspondence: Jerzy A. Walocha, Chair of Anatomy, Medical College of the Jagiellonian University, ul. Kopernika 12, 31–034 Kraków, Poland, tel: +48 12 422 95 11, fax: +48 12 430 30 06; e-mail: mburbani@cyf-kr.edu.pl

tion of 50%. Next the specimens were diaphanised in a mixture of absolute alcohol and methyl salicylate (proportions 3:1, 1:1, 1:3) and then they were diaphanised in pure methyl salicylate. The whole specimens were next analysed under a light microscope (PZO type Mst-130), using 5 to 80 × magnification. At 25 × magnification the scale was an equivalent of 14.5 μm.

Next, successive slides of the myomatous uteri injected with acrylic emulsion were prepared. Excisions were taken from the prepared material after its dehydration in rising concentrations of ethyl alcohol and immersion in methyl benzoic and benzene. The slides were immersed in paraffin and cut into fragments 4 μm wide. Next they were dyed with haematoxilin and eosin. The material was studied under the light microscope using the magnification 5 to 40 ×.

A few prepared slides were deparaffinised, hydrated and prepared to be tested for von Willebrandt's factor. The primary antibody, anti Human von Willebrandt factor (Dako company) and EnVision complex + HRP/MO, was used for immunochistochemical reaction (ratio 1:50). Next the antigen (cells of the vascular endothelium) was unveiled with the help of citrate buffer (pH 6.0) in the microwave (750 W). Carbazole was applied in colour reaction. The material was studied under the microscope at magnifications between 20 and 330 ×. The results were documented with the help of photographs.

RESULTS

Uterine leiomyomas are the most common benign tumours of the female internal genital organs [3, 6]. Acrylic emulsion Liquitex R does not penetrate through the capillary bed because of the size of its molecules and high viscosity (Fig. 1, 2). It is well observable in typical histological specimens (Fig. 3) and slides showing the immunochistochemical reaction for von Willebrandt's factor. High magnifications of the transverse sections revealed the network of small blood vessels, usually not filled with acrylic emulsion (Fig. 4), but visible in the immunochistochemical reaction [22].

DISCUSSION

Injection of isolated adult human organs can be complicated with impediments dictated by delay, which must occur between the time of death and the moment of procedure preparing the material for research [1, 2, 16, 21, 22]. The walls of the vessels become more permeable due to possible necrosis. That is why when we carry out the studies on the dissection material, a certain part of it is eliminated during the initial stage of research. However such studies should be undertaken due to the number of

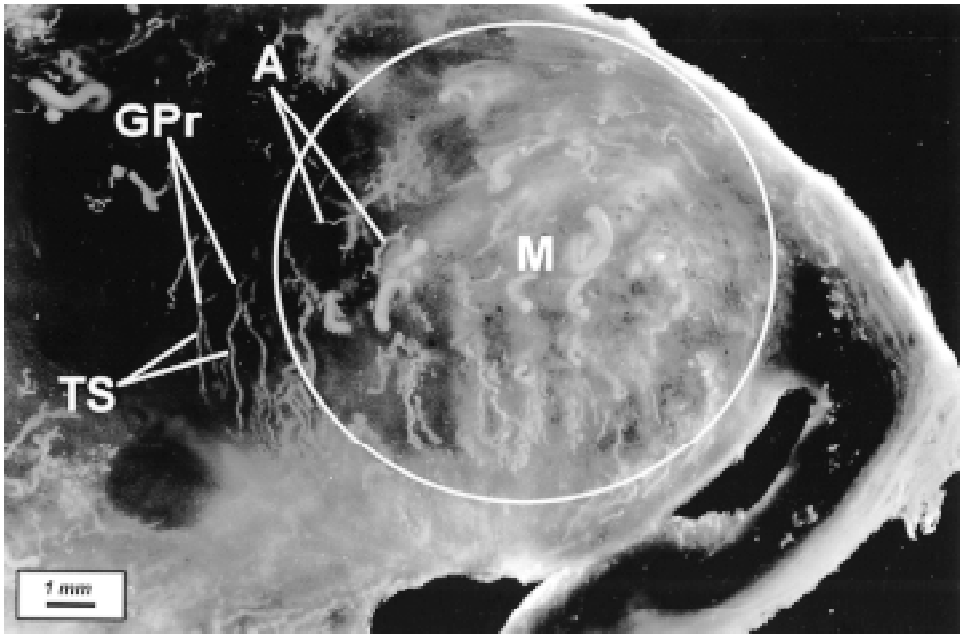

Figure 1. Uterus of 50-year-old female with subserous leiomyoma. Transverse section of specimen injected with water solution of acrylic emulsion Liquitex R (Binney and Smith, USA), diaphanised in methyl salicylate; GPr — radial arteries, TS — spiral arteries, M — leiomyoma, A — arterioles.

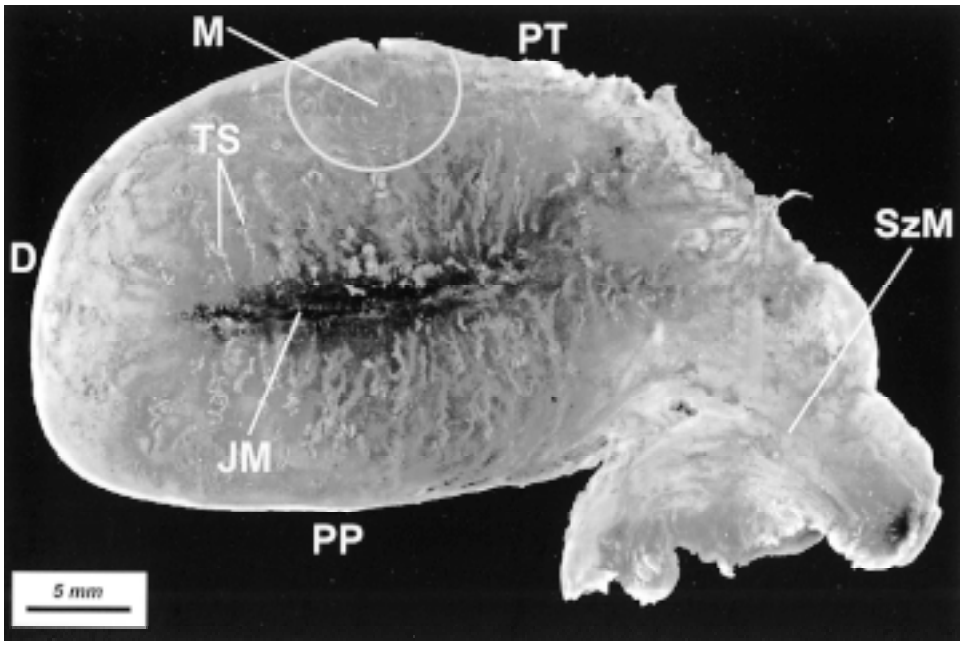

Figure 2. Uterus of 41-year-old female with small subserous leiomyoma. Sagittal section of specimen injected through the uterine arteries with water solution of acrylic emulsion Liquitex R (Binney and Smith, USA), diaphanised in methyl salicylate; D — fundus of the uterus; M — leiomyoma, PT — posterior surface, PP — anterior surface, JM — uterine cavity, TS — spiral arteries, SzM — uterine cervix.

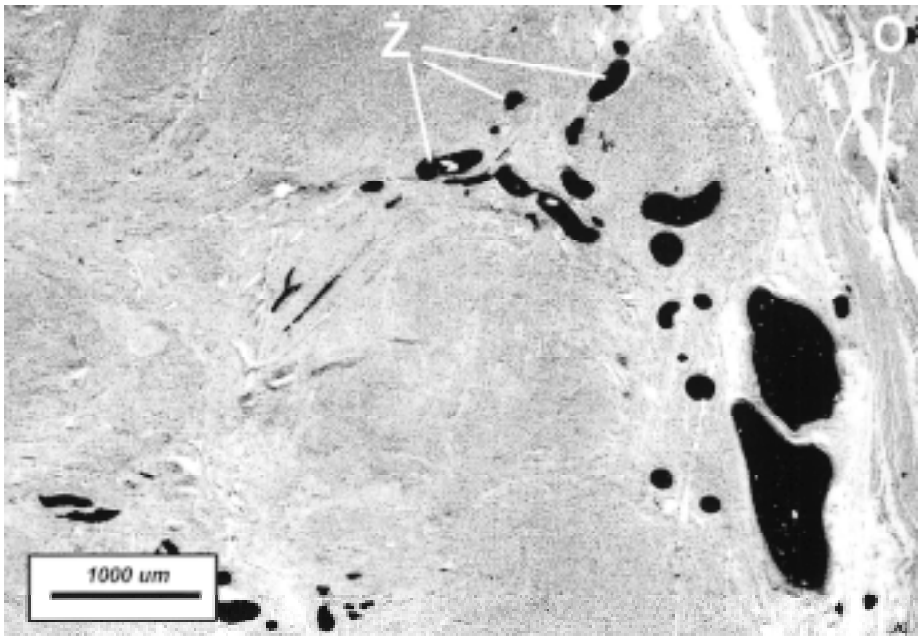

Figure 3. Uterus of 71-year-old female. Margin of the leiomyoma. Uterine veins injected with water solution of acrylic emulsion Liquitex R (Binney and Smith, USA), dyed with haematoxilin and eosin; O — margin of the leiomyoma, Ż — small venous vessels penetrating the tumour.

valuable studies that were published, based on the observations of injected vascular beds of human foetuses as well as of the dissection material obtained from adult human cadavers, which was different from adult human uteri [16, 18, 19]. Several studies of vasculature of different human organs using acrylic emulsion Liquitex R [18, 19] or other types of dyes [1, 2, 5, 7–13, 15, 20, 21] were carried out but none of them was carried out with the help of immunohistochemical reaction.

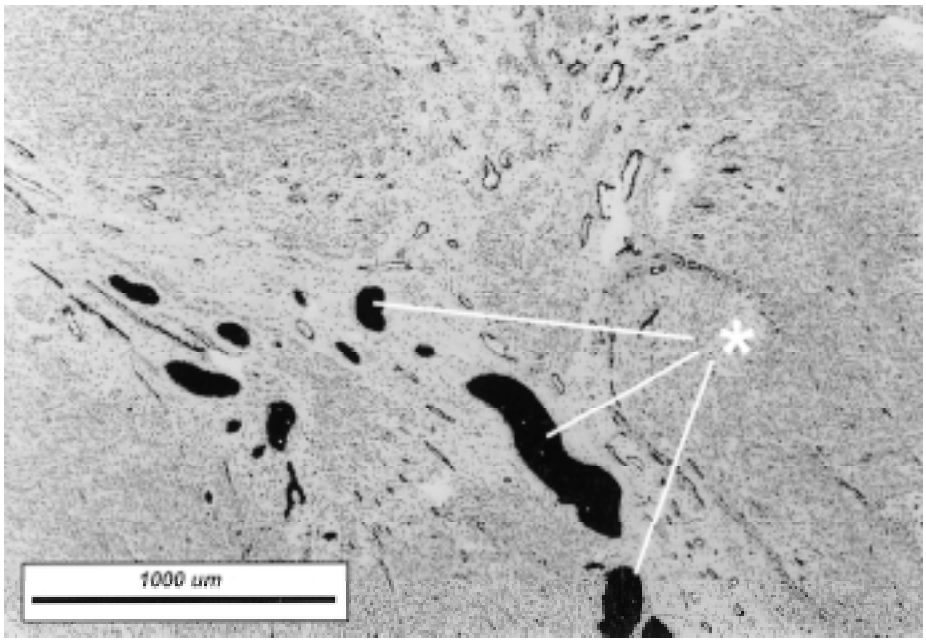

Figure 4. Uterus of 51-year-old female. Leiomyoma. Arteries (*) injected with water solution of acrylic emulsion Liquitex R (Binney and Smith, USA). Immunohistochemical test for von Willebrandt's factor.

CONCLUSIONS

Water solution of acrylic emulsion Liquitex R (Binney and Smith, USA) is an advisable and useful filling mass, used in studies of the vascular system of human organs. Limitations resulting from lack of penetration through the capillary bed can be compensated by classic histopathological research and immunohistochemical test, i.e. application of antibodies binding von Willebrandt's factor, which can be visualised with the help of an appropriate colour reaction.

ACKNOWLEDGEMENTS

The authors wish to thank Dr Jan Kołodziej, MD, PhD from the Forensic Medicine Institute of the Medical College of the Jagiellonian University for help in the collection of dissection material.

REFERENCES

1. Aleksandrowicz R, Czerkwiński J (1981) The valves of the uterine and ovarian veins. Verh Anat Ges, 75: 731.
2. Aleksandrowicz R, Czerkwiński J (1981) The veins of the female reproductive system. Fol Morphol Českoslov, 29 (3): 227–229.
3. Buttram VC Jr, Reiter RC (1981) Uterine leiomyoma: etiology, symptomatology and management. Fertil Steril, 36: 433–447.
4. Cole FJ (1921) The history of anatomical injections. In: Singer C (ed.). Studies in the history and methods of science. Oxford University Press, London, pp. 285–343.
5. Czerkwiński J (1980) Żyły i zastawki żylne wewnętrznych narządów płciowych kobiet w normie i niektórych stanach chorobowych. Pch D Thesis, Warszawa.
6. Dixon D, Flake GP, Moore AB, He H, Haseman JK, Risinger JI, Lancaster JM, Berchuck A, Barrett JC, Robboy SJ (2002) Cell proliferation and apoptosis in human uterine leiomyomas and myometria. Virchows Arch, 441 (1): 53–62.
7. Farrer-Brown G, Beilby JO, Tarbit MH (1970) The vascular patterns in myomatous uteri. J Obstet Gynaec Brit Comm, 35: 967–975.
8. Farrer-Brown G, Beilby JO, Rowles PM (1970) Microvasculature of the uterus. An injection method of study. Obstet Gynec, 35: 21–30.
9. Farrer-Brown G, Beilby JO, Tarbit MH (1971) Venous changes in the endometrium of myomatous uteri. Obstet Gynec, 38: 743–751.
10. Faulkner RL (1944) The blood vessels of the myomatous uterus. Am J Obstet Gynec, 47: 185–189.
11. Faulkner RL (1945) An injection study of uterine blood vessels. Am J Obstet Gynec, 49: 1–6.
12. Faulkner RL (1951) An injection study of the blood vessels of the bleeding uterus. Am J Obstet Gynec, 61: 766–769.
13. Holmgren B (1938) Some observations on the blood vessels of the uterus under normal conditions and in myoma. Acta Obstet Gynaec Scand, 18: 192–203.
14. Kuś J (1969) Historia metod iniekcyjnych w naukach morfologicznych. Folia Morphol (Warsz), 28 (2): 147–160.
15. Lindenbaum E, Brandes JM, Itzkowitz J (1978) Ipsi- and contralateral anastomosis of the uterine arteries. Acta Anat, 102: 157–252.

16. Martín-Orti R, Stefanov M, Gaspar I, Marin R, Martin--Algualcil (1999) Effect of anticoagulation and lavage prior to casting postmortem material with Mercox® and Batson®. J Microsc, 195: 150–160.
17. Miodoński AJ, Kuś J (1978) Metody iniekcyjne w badaniach układu krwionośnego. Wszechświat, 7–8: 183–186.
18. Pityński K, Skawina A, Lipczyński W, Polakiewicz J, Walocha J (1996) The posterior gastric and superior polar arteries in human fetuses. Folia Morphol (Warsz), 55 (1): 43–49.
19. Pityński K, Skawina A, Polakiewicz J, Walocha J (1998) Extraorganic vascular system of adrenal glands in human fetuses. An Anat, 180: 361–368.
20. Sampson JA (1913) The influence of myomata on the blood supply of the uterus with special reference to abnormal uterine bleeding. Surg Gynaec Obstet, 16: 144–168.
21. Schlegel JU (1946) Arteriovenous anastomoses in the endometrium in man. Acta Anat, 1: 284–325.
22. Walocha JA (2001) Unaczynienie mięśniaków macicy. Pch D Thesis, Kraków.