FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol.13, No 3, 2006, pp. 181 - 184 UC 340.6:612.46

IDENTIFICATION OF POSTMORTEM AUTOLYTIC CHANGES ON THE KIDNEY TISSUE USING PAS STAINED METHOD

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Summary. *On the sample of 112 experimental rats a PAS histochemical analysis of renal cortex specimens has been done. The animals were kept, after the sacrifiction, at the temperatures of 10°C, 20°C and 30°C. The rats have been dissected from 1 to 72 h. after the sacrificing. After the standard procedure of tissue processing the samples would be stained with PAS, then, the following structures would be analyzed: glomerular mesangium, glomerular basal membrane, parietal layer of Bowman's capsule, tubular basal membrane and apical parts of tubular epithelial cells. The results of this research confirm that the dynamic of the appearance of morphological post mortal autolytic changes depends on the time of death and on the temperature at which the autolysis is evolving and also, that there is a certain autolytical order depending on the environmental conditions.*

Key words: *Autolysis, postmortem changes, renal glomeruli, kidney, PAS stained*

Introduction

Forensic medicine practice shows continuous expert, scientific, ethical, and legal need to, besides identifying the cause of death, we identify the time of death as precise as possible. The most common method for establishing the postmortem time is based on the development of corpse's characteristics and changes which depend on variable factors such as the temperature, air moisture and the surroundings (1,2). During last decades many postmortem interval studies referring to the establishing of biochemical, histological, histochemical and ultra structural changes in different tissues and organs were done (3-6).

This problem had been seen as a forensic one for a long time, but later, especially in the second half of the last century it became more important due to the use of new treatment methods in modern medicine referring to the tissue and organ transplantations. That's why the determination of the time of death problem in the current level of medical science and technique became more popular, not only for the forensics, but also for some other biomedical specialties: surgeons, nephrologists, traumatologists, hematologists, transplatators, etc. (7). Until now, separately shown, some scientifically valuable results show certain pattern of the appearance and development of certain autolitical changes in function of time passed from the moment of death, but haven't been confirmed in forensic practice as precise and optimal as the solution to this problem, so this work is written in order for a complex of effective and reliable methods and criteria for this important part of biomedical sciences to be established.

The aim of this study is the determination of order and certain pattern of changes in kidney tissue during postmortem time interval by using the PAS method of coloring under certain conditions. Quality and quantity changes are expected, proportional to the interval length and certain external conditions, which would represent a significant income to the complex of autolitical changes establishment for the precise determination of the time of death.

Materials and Methods

The research is done on 112 experimental animals-rats, Wister race, weight 180-200 gr., aged 4-6 months, equal number of both sexes, killed by choking. Four animals, two male and two female, were dissected right away, as a control group. The rest 108 rats were classified into 3 equal groups, each containing 36 animals, and kept at the temperature 8-10°C (temperature group 1), 18-20°C (temperature group 2) and 28-30°C (temperature group 3), on the air-moisture of 70%. Inside of each temperature group, the time subgroups were determined, depending on the time passed from the sacrificing to the autopsy. In each of the subgroup, 4 rats were dissected in 1, 2, 4, 6, 12, 24, 36, 48, 72 hours after killing. During the autopsy, a part of a kidney were fixated in a 10% foramaldexyde solution, and processed in an autotechnicon. Paraffin sections 5μm thick, was stained using the PAS method (basic fuchsin). The analysis and the microscopic view of the prepared remedies were done on the "OLYMPUS BX 50" micro-

scope, with the automatic photo camera used for photographing chosen fields of the remedies.

Results

By means of light microcopy of the kidney sections treated with PAS method morphological changes in glomerular mesangium, glomerular basal membrane, parietal layer of Bowman's capsule, tubular basal membrane and apical parts of tubular epithelial cells. Morphological changes are shown in table 1.

Temperature group 1

During the first and second hour of post mortal autolysis at animals kept at 8-10°C PAS positive reaction in all examined kidney structures.

In fourth hour of postmortal autolysis, in this temperature group, a rare, scattered separation of PAS positive apical proximal tubule cell parts, as a scattered putting out of tune their basal membranes. On the other structures PAS positivity is reflected.

During the sixth hour, PAS positive reaction of mesangium, tubular basal membranes, and blood vessels is still recognizable, and is missing in certain areas of tubule and a lower PAS reaction intensity on basal tubule membranes were noticed. PAS positive apical cell tubule parts show a sporadic separation and their presence in tubule luminary.

Reduced PAS positive reaction during the 12^{th} and 24^{th} hour of autolysis is noticeable in tubular basal membranes and in apical parts of epithel tubule cells at those torn apart and located in luminary, and also at those at their own places. In the places of missing apical parts a small granular content with few PAS positive fragments. Mesangium and glomerular basal membrane during this hour still shows clear PAS positivity. The characteristic of 36-72 hours of autolysis is still the reduction of the PAS positivity of mesangium and glomerular basal membrane, but all examined structures keep the PAS positivity till the end of examination.

Temperature group 2

The expressive PAS positive reaction during the first hour at the kidneys of the animals kept postmortem at the temperature 18-20°C show all examined structures.

Except for scattered, starting separation of PAS positive tips of the proximal tubule epithelial cells of the cortex, in the second hour of post mortal analysis other changes in relation to the previous group (Fig. 1) weren't noticed.

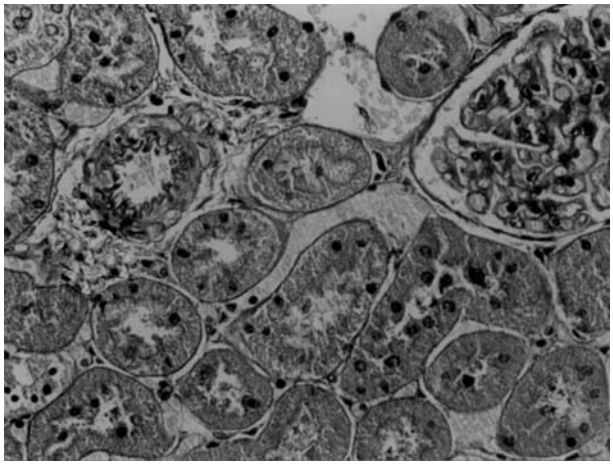

Fig. 1. Kidney cortex II temperature group on 2 hour after death (PAS ×300)

During the fourth, and a little more expressive during the sixth hour of autolysis can be noticed that, basal tubule membranes, especially in the cortex are partly less PAS positive in relation to the previous groups. The other parts do not show greater differences.

The characteristic of the 12^{th} hour of the autolysis in this temperature group are lowered PAS positivity of glomerular mesangium and a starting less expressive positive reaction on apical parts of tubule cells. On the other observed structures a clear PAS positive reaction is still maintained (Fig. 2).

Table 1. Morphological changes of the kidney sections treated with PAS method

	Mesangium			Basal membrane of glomeruli			Basal membrane of Bowman's capsule			Tubular basal membrane			Apex of cell		
h	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1.	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++o
2.	++	++	++	++	++	++	++	++	++	++	++	+	++	++o	++
4.	++	++	++	++	++	++	++	++	++	++	+	+	++o	++	+
6.	++	++	+	++	++	+	++	++	+	+	+	+ –	+	++	+
12.	++	+	+ –	++	++	+	++	++	+	+	+	– +	+	+	– +
24.	++	+	– +	++	+	+ –	++	+	+ –	+	+ –	– +	+	+ –	– +
36.	+	+ –	–	+	+	+ –	++	+	+ –	+	– +	– +	+	+ –	–+
48.	+	– +	–	+	+	– +	++	+ –	+ –	+	– +	–	+	– +	– +
72.	+	–	–	+	+ –	–	++	+ –	– +	+ –	–	–	+	– +	–

++ expressive PAS positivity
+ bright PAS positivity
+ – poor PAS positivity
– + hardly noticeably PAS positivity
– absence PAS positivity
++o separation of PAS positive apical cell parts

I – 10°C
II – 20°C
III – 30°C

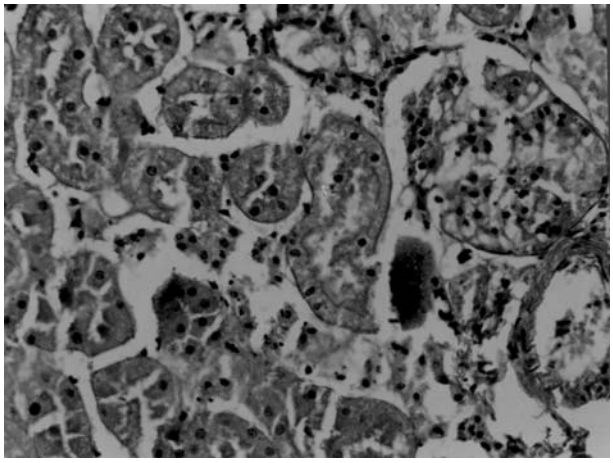

Fig. 2. Kidney cortex II temperature group on 12 hour after death (PAS ×300)

By following the coloring intensity of mesangium in the further time research, a gradual lowering of the positivity until 48th hour is noticeable, and in 72nd hour mesangium becomes negative. The identical order of changes is noticeable on the basal tubule membrane. The positivity of glomerular basal membrane, Bowman's capsule and apoical parts of the epithelial cells, during this time interval gradually decreases, but remains till the end of the time examined.

Temperature group 3

During the 1st hour of post mortal autolysis, at the animals kept at the temperature of 28-30°C after the sacrificing, the observed structures show a clear positive PAS reaction. The only change can be seen at scattered and rare tearing of apical parts of tubular epithelium.

Except for a starting weakening of PAS positive reaction on basal tubule membranes, primarily in the cortex area, with the rest of the structures, at the end of the 2nd hour, significant changes in relation to the previous group are unnoticeable.

The 4th hour of the autolysis as characterized by a reduced PAS positive reaction on tubular basal membranes and apical arts of epithel tubule cells. On the other structures positive PAS reaction is clearly visible.

During the 6th hour of the autolysis, a weak PAS positivity of tubular basal membranes is evident, while the above mentioned is slightly clearer, but still weak in relation to the previous time group on glomerular mesangium, glomerular basal membrane and parietal layer of Bowman's capsule.

A weak PAS positive reaction in mesangial space can be seen during the 12th hour of autolysis. Parietal layer of Bowman's capsule, glomerular basal membrane and blood vessel basal membrane keep a clear PAS positive reaction (Fig. 3).

From the 24th hour the PAS positivity of mesangium decreases and the above mentioned becomes negative during the 36th hour. The basal membrane of the glomerul, tubule and apical epithelial cells become the PAS negative during the 72nd hour, while parietal layer of Bowman's capsule keeps barely noticeable positivity till the end of the time examined.

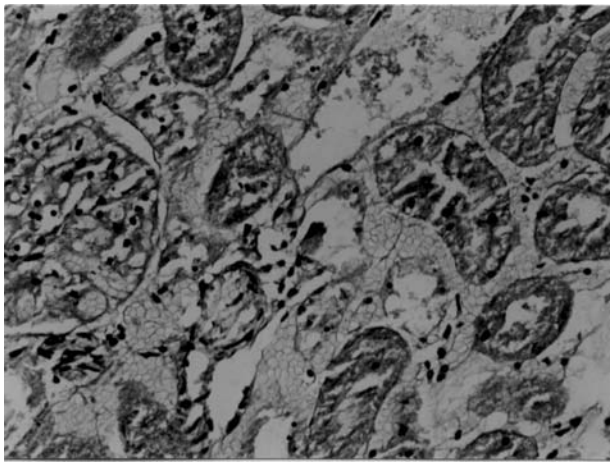

Fig. 3. Kidney cortex III temperature group on 12 hour after death (PAS ×300)

Discussion

Hystomorphologically, the autolysis represents the intravital or post mortal disintegration of living structures, and biochemically corresponds to a loss in the system of metabolic balance with demotion of the metabolic substance which results in energy and material loss. Autolysis matches with the activity of certain enzymes, called autolytical enzymes, proved in lysosomes of living cells, which after death lead to the destruction of their own cell components. Those enzymes disintegrate intracellular material, including organelles very quickly, so the cytoplasm becomes of homogenic looks and intensively eosynophilic, which culminates with a loss of cell details and tissue architecture (3,8,9,10).

First changes of the PAS positive structures in kidney tissue are reflected in the separation of apical parts of the tubule epithel, which already appears during the 1st hour at 20°C, and the 4th hour at 10°C. A reducing of the apical structures and tubular basal membranes' PAS positivity at the temperature o parietal layer of Bowman's capsule f 30°C, 4 hours after death is noticeable. This is evident in the temperature group 2 as well, while the other structures still keep clear PAS positivity. In the 48 hours after death, in the temperature group, the apical parts keep barely noticeable PAS positivity, which is completely lost in the 72nd hour postmortem, in correlation with other authors (11,12).

Glomerular mesangium shows a clear PAS positivity in all temperature groups until the 6th hour. In the temperature group 3 from that period a gradual decrease begins, so this structure is slightly positive in the 12th hour, in the 24th hour barely noticeable, and from 36th hour postmortem PAS positivity is completely gone. The same change progression occurs in the temperature group 2, where the decrease of the PAS positivity is noticeable during the 36th, barely noticeable in 48th, and completely lost in 72nd hour after death.

The PAS positivity oglomerular basal membrane is kept at 10°C the whole time, and after 36 hours a slight decrease of the color intensity occurs. In the temperature group 2, the PAS positivity is clearly noticeable till the 12th hour after death. The reducing begins in the 24th hour, and at the end of the 72nd hour it is still barely present. At the temperature of 30°C the intensity of the PAS positive changes starts degreasing in the 6th hour, it is barely noticeable in the 48th hour, and completely absent in the 72nd hour of post mortal autolysis, which is in accordance with the researches done by the other authors (2,13,14).

Basal membrane of parietal layer of Bowman's capsule keeps he PAS positivity for the longest period of time because its reduction in the temperature group 3 begins in the 24th hour, maintains till the end of the examination and represents the only structure that keeps PAS positivity at this time and temperature. In the other temperature groups this kidney structure shows good reception to this color, almost till the end of the examination.

In all temperature groups it is noticeable that the PAS positivity starts reducing first on the tubular basal membrane, and in the temperature groups 2 and 3 it is completely absent at the end, and in the temperature group 1 barely expressed, which is accordance with the results of the other authors (11,14,15).

Conclusion

The results of this research confirm that the level of expression and the dynamic of the appearance of morphological post mortal autolytical changes in the kidney tissue depend on the time of death and on the temperature at which the autolysis is evolving and also, that there is a certain autholitical order depending on the environmental conditions.

This represents a significant contribution to the exact determination of the time of death.

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IDENTIFIKACIJA POSTMORTALNIH AUTOLITIČKIH PROMENA U TKIVU BUBREGA PRIMENOM PAS METODE

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Kratak sadržaj: *Na uzorku od 112 eksperimentalnih životinja-pacova Wistar soja, izvršena je morfološka analiza isečaka bubrega primenom PAS metode u cilju otkrivanja stepena i brzine razvoja postmortnih autolitičkih promena. Životinje su podeljene u 4 osnovne grupe i to u kontrolnu grupu, čiji su isečci uzimani odmah nakon žrtvovanja, i u tri po broju jednake grupe životinja koje su nakon žrtvovanja čuvane na temperaturama od 10℃, 20℃ i 30℃. U okviru svake temperaturne grupe, odredjeno je devet vremenskih podgrupa, i u svakoj od njih su secirana po četiri pacova na 1, 2, 4, 6, 12, 24, 36, 48 i 72 sata po usmrćenju. Isečci bubrega su nakon standardne obrade bojeni PAS metodom pri čemu su analizirane sledeće strukture: mezangijum glomerula, bazalna membrana glomerula, bazalna membrana parijetalnog lista Bowman-ove kaspule, bazalna membrana tubula i apikalni delovi tubularnog epitela. Rezultatima ovog istraživanja potvrdjeno je da su stepen izraženosti i dinamika ispoljavanja morfoloških postmortnih autolitičkih promena u direktnoj zavisnosti od dužine postmortnog vremena i temperature spoljašnje sredine pri kojoj se autoliza odvija.*

Ključne reči: *autoliza, postmortne promene, glomerul, bubreg, PAS metoda bojenja*