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Fatty degeneration in renal tissue in cases of fatal accidental hypothermia

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Abstract: The expression of morphological findings in cases of death due to hypothermia varies from case to case. The characteristics mainly depend on the difference between the body core temperature and the air or water temperature as well as the velocity of cooling. The diagnosis of fatal hypothermia is typically based on macro-morphological findings such as Wischnewsky spots and erythema of the skin of the large joints in combination with the circumstances of death. One reported micro-morphological finding of premortal hypothermia is cellular vacuolization of the renal tissue. Therefore, fatty degeneration of renal tissue was investigated in a study group which included 41 cases of death due to accidental fatal hypothermia and a control group of 45 deaths without known hypo- or hyperthermal temperature exposure prior to death. Fatty degeneration was found in the renal tubular epithelium as well as in the capillary loops of the glomeruli. In the study group 34 cases (82.92 %) presented a positive Sudan reaction with an intensive expression. In contrary, only 20 cases (44.44 %) of the control group stained positive, the majority of which (95.0 %) only in a weak graduation. Fatty degeneration of renal tissue (tubular epithelium as well as glomeruli) seems to be a reliable diagnostic histopathological marker of hypothermia. A correlation between the expression of renal fatty degeneration and the expression of macro-morphological signs such as Wischnewsky spots or cold erythema of the skin could not be found.

Key Words: Fatty degeneration, hypothermia, kidney, Sudan staining, vacuolization.

INTRODUCTION

The diagnosis of fatal hypothermia is typically based on macro-morphological findings such as gastric hemorrhagic erosions and erythema observed on the skin of the large joints in combination with the circumstances of death.

The extent of morphological traits in cases of death due to hypothermia varies from case to case. When fatal hypothermia occurs in an environment of dry air macroscopical findings proving death due to hypothermia are found in only about 2/3 of cases [1, 2]. The manifestation of the former mainly depends on the difference between body core temperature and ambient air temperature, or, in cases of immersion hypothermia, water temperature. The longer the time period in which the body core temperature drops, the more intense will the alterations in organ tissues be. Consequently, in cases where the body core temperature cools down quickly, morphological traits of pre-mortal hypothermia can be missing entirely, especially in cases of immersion hypothermia. In cold water a body cools down 20 to 25 times faster than on land even if the ambient air is the same temperature [1].

Extensive research has been performed on histopathological alterations of organs after vital hypothermic exposure. Reported micro-morphological alterations with an emphasis of cellular vacuolization were reported in:

the hormone producing cells of the anterior lobe of the pituitary gland [3-7],

myocytes and hepatocytes [1, 8, 9],

pancreatic cells [10] and

the renal tubular epithelium [11].

In general, cellular vacuolization can be caused by cytoplasmic lipid or glycogen deposits. These two

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phenomenons can be differentiated microscopically by using different staining methods: Sudan staining for lipids and PAS-staining for glycogen.

Besides the initial report of Thrun on fatty vacuoles in renal tissue [12] only one lager study has been published on that subject so far [11] making our study an important addition. The aim of the present study was to investigate the levels of steatosis in renal vacuolization in cases of fatal hypothermia. We investigated cases of fatal hypothermia as well as a large spectrum of different causes of death without premortal thermal influences.

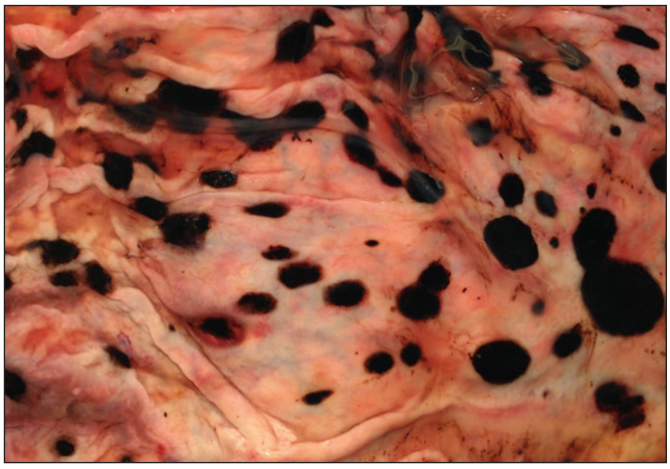

Figure 1. Wischnewsky spots in the gastric mucosa in case of fatal hypothermia.

MATERIAL AND METHODS

The study group included 41 cases of death due to accidental hypothermia (Table 1). In all cases an autopsy was performed at the Institute of Legal Medicine in Bonn, Germany. The group included 27 females and 24 males between 27 and 88 years of age (mean age 62.92 years).

In 30 cases death occurred outdoors, in 11 cases indoors, respectively. In all cases typical macro-morphological findings leading to the diagnosis of fatal hypothermia were present. Wischnewsky spots were found in 37 cases (90.24%). Typical cold erythema of the skin were present in 14 cases (34.14 %) (Fig. 1). Paradoxical undressing was found in 9 cases (24.47%). The combination of Wischnewsky spots and cold

Table 1. Study group, F: female, M: male, W: Wischnewsky spots, E: erythema, PU: paradoxical undressing

No.	Age	Sex	W	E	PU	Place of discovery	Sudan stain positive	Sudan stain Grade
1	79	M	X			Indoor	X	2
2	44	M	X			Indoor	X	3
3	76	M	X			Outdoor		0
4	43	M	X			Indoor	X	2
5	58	M	X	X		indoor		0
6	71	M			X	Outdoor	X	3
7	84	M	X		X	Indoor	X	2
8	43	M	X			Indoor	X	3
9	76	F	X			Indoor	X	3
10	44	M			X	Outdoor	X	2
11	44	F	X			Outdoor		0
12	59	M	X	X		Outdoor	X	1
13	44	M	X		X	Indoor	X	3
14	63	M	X	X		Indoor	X	1
15	88	F		X		Outdoor		0
16	78	M	X			indoor	X	1
17	58	F	X			indoor	X	3
18	55	F	X			Outdoor	X	1
19	67	F	X	X		Indoor	X	3
20	86	F	X	X		Indoor	X	2
21	62	F	X			Indoor	X	2
22	73	M	X			Indoor	X	1
23	74	F		X		Outdoor		0
24	71	M	X	X		Indoor	X	1
25	27	M	X			Indoor	X	1
26	76	M	X	X	X	Outdoor	X	1
27	75	F	X			Indoor	X	1
28	30	M	X		X	Outdoor	X	3
29	81	F	X			Indoor	X	1
30	71	F	X			Indoor	X	3
31	54	M	X	X		Outdoor		0
32	59	F	X	X	X	Indoor	X	1
33	58	M	X		X	Indoor	X	1
34	53	M	X			Indoor	X	3
35	75	M	X		X	Indoor	X	2
36	74	F	X			Indoor		0
37	60	F	X			Indoor	X	2
38	43	M	X	X		Indoor	X	2
39	85	F	X	X		Indoor	X	3
40	49	M	X	X		Indoor	X	2
41	70	F	X			Indoor	X	3

erythema was present in 12 cases (29.26%). All three characteristics of hypothermia were found in only 2 cases (4.87%).

The control group comprised 45 deaths without known hypothermal or hyperthermal exposure prior to death. In this group we included 20 females and 25 males ranging from 7 to 100 years of age with a mean age of 59.84 years. This group included cases with natural and non-natural manner of death and a broad spectrum of acute, subacute and prolonged causes of death (Table 2).

During forensic autopsies renal tissue samples have been taken. The samples were fixed in 8–10 % formalin. After fixation the tissue was embedded in paraffin wax and sectioned (3–4 μm). In every case Sudan staining (Herxheimer Sudan III and IV) was performed. Formalin fixed tissue samples were frozen and sectioned. The nuclei were stained with hemalaun. Additionally, all samples were stained with hemalaun-eosin (H&E).

Every slide was examined by light microscope at a 200x magnification in 20 visual fields. The extent of each staining was rated semiquantitatively in a three-degree scale. The number of positively red-orange stained lipid droplets in relation to all studied structures/cells in the renal tissue which were visible in each visual field was estimated on a percentage basis [11].

All kidneys were analyzed by using a light microscope and the results of the study group and the control group were compared.

RESULTS

Steatosis was found in the renal tubular epithelium as well as in the capillary loops of the glomeruli.

In the study group 34 cases (82.92%) presented a positive Sudan reaction. 12 of these positive cases (35.29%) showed a weak reaction (grade 1), whereas in 10 cases (29.41%) a moderate reaction (grade 2) and in 12 cases (35.29%) an intensive reaction (grade 3) was found.

Table 2. Control group, F: female, M: male, Cause of death and Graduation of Sudan Staining according to Table 3

No.	Age	Sex	Cause of death	Sudan stain positive	Sudan stain Grade
1	59	M	Myocardial infarction	X	1
2	100	M	Pneumonia and enteritis	X	1
3	85	F	Sepsis		0
4	69	M	Heamorrhage due to polytrauma	X	1
5	73	F	Pulmonary embolism	X	1
6	72	F	Heamorhage after rectum resection	X	1
7	34	M	Sepsis		0
8	22	M	Heamorrhage due to polytrauma		0
9	30	M	Drug intoxication		0
10	67	F	Pericardial tamponade to aortic dissection		0
11	68	F	Pulmonary embolism		0
12	64	F	Drowning	X	1
13	80	F	Myocardial infarction		0
14	83	F	Subdural haemorrhage		0
15	75	F	Ligature strangulation		0
16	88	M	Pulmonary oedema	X	1
17	49	F	Cerebral heamorrhage		0
18	69	M	Heamorrhage (suicide)		0
19	47	M	Drug intoxication		0
20	82	F	Coronary insufficiency		0
21	49	M	Coronary insufficiency	X	1
22	47	M	Pulmonary embolism		0
23	74	M	Myocardial infarction	X	1
24	38	M	Drug intoxication		0
25	95	M	Myocardial infarction	X	2
26	80	F	Coronary insufficiency		0
27	7	F	strangulation		0
28	69	F	Pulmonary embolism	X	1
29	44	M	Myocardial infarction	X	1
30	53	F	Pulmonary embolism		0
31	87	F	Haemorrhage	X	2
32	84	F	Coronary insufficiency	X	1
33	44	M	Cardiomyopathy	X	2
34	30	M	Drug intoxication		0
35	44	M	Coronary insufficiency	X	1
36	31	M	Drug intoxication		0
37	73	M	Heamorrhage		0
38	76	M	Myocardial infarction	X	1
39	63	F	Stroke		0
40	30	M	Intracranial heamorhage		0
41	46	F	Heamorrhage by esophageal ulcer	X	1
42	76	F	Hypoxic cerebral damage after false intubation	X	1
43	51	M	Myocardial infarction	X	1
44	25	M	Drug intoxication		0
45	61	M	Cardiomyopathy		0

In the control group, only 20 cases (44.44%) stained positive. 19 of these cases (95.0 %) exhibited grade 1 and only 1 case (5.0%) grade 2.

The result of the chi-square test indicates a significant difference ($p < 0.001$) of the amount of positive stainings between both groups. Not only the number of cases with fatty degeneration varies between both groups, also the intensity of staining varies. In the control group the majority of cases presented a weak staining reaction, whereas in the study group the majority presented a more intensive staining than grade 1. Furthermore, grade 3 could never be observed in the control group.

The results are shown in Table 1 (study group) and Table 2 (control group) as well as in Figs 7 and 8. As shown in Table 1 there is no correlation between the expression of renal tissue fatty degeneration and the expression of macro-morphological signs like Wischnewsky spots or cold erythema of the skin. Specifically cases no. 5 and 31 did not show fatty degeneration of renal tissue, but presented both, Wischnewsky spots as well as erythema.

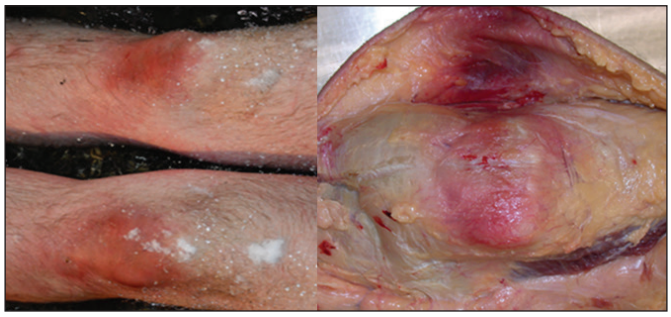

Figure 2. Erythma of the skin (right: subcutaneous tissue of the knee) in case of fatal hypothermia.

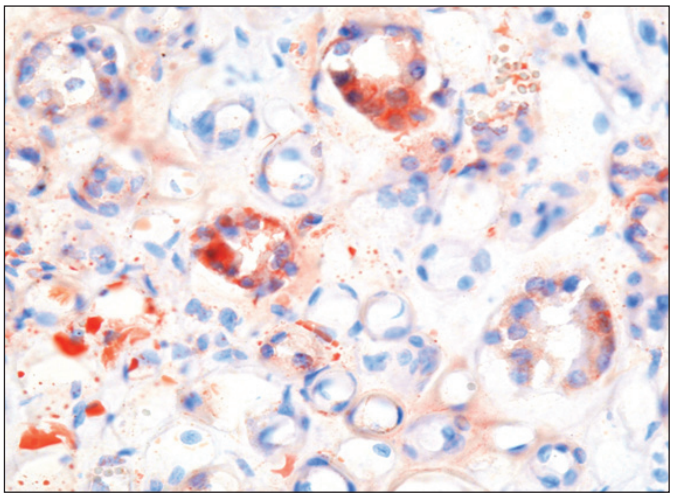

Figure 3. Renal tissue, study group, grade 1, Sudan stain, 200x.

In cases 6 and 10 macro-morphological signs were missing, but a high level of fatty degeneration was present.

DISCUSSION

The so called Armanni-Ebstein phenomenon of glycogen deposits in renal tubular epithelium is defined as swollen and optically empty cells that are typically seen in diabetic patients with disturbance of the blood glucose level after a diabetic coma or ketoacidosis [13-16]. Fatty deposits in the cytoplasm of renal tubule epithelium are found in case of ketoacidosis and diabetic coma as well as in hypothermia [13-15, 17]. They should be addressed as subnuclear lipid containing vacuolization instead of classical Armanni-Ebstein lesions [16]. The degree of fatty degeneration has obviously a strong correlation with the ketone body concentration [15, 17].

Hypothermia is defined as a body core temperature less than 35 °C [1, 2]. In the majority of cases hypothermia occurred accidentally, usually in the context of acute health impairments or intoxications paired with a state of unconsciousness or helplessness. Central depressive substances can have a direct negative effect on the body's temperature regulation and can significantly promote hypothermia. Usually, the suspicion of death due to hypothermia results from the circumstances of the body's location, i.e. outside and unsheltered, or in a non-heated apartment in cold weather or paired with the phenomenon of paradoxical undressing and hiding [7]. Autopsy may then proof the suspicion of hypothermic influences.

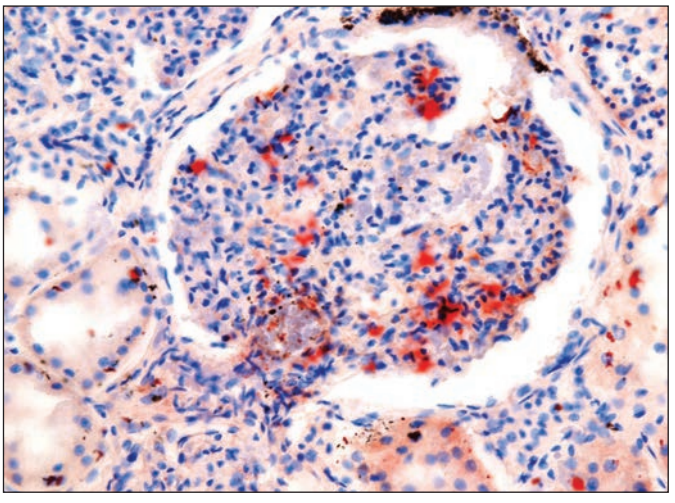

Figure 4. Renal tissue, glomerulum, study group, grade 1, Sudan stain, 400x.

Table 3. Graduation of the hsp and Sudan-staining (according to 11)

Distribution on a percentage basis	Graduation	Explanation
0	Grade 0	No staining
> 0 bis 29.99 %	Grade 1	Weak staining
30 bis 59.99 %	Grade 2	Moderate staining
60 bis 100 %	Grade 3	Intensive staining

In general, fatty vacuolization is caused by a disturbance of the cellular lipid metabolism. Free fatty acids are energy sources and if needed mobilized from stored triglycerides in adipose tissue and transported to i.e. hepatocytes. After fatty acid transport in the cells, fatty acides are mainly used to rebuild triglycerides, but also for the production of ketone bodies, phospholipids and cholesterol esters (lipoproteins). The resynthesised tryglycerides are bound to liproptoteins and can be exported from the cell. In case of a disoder in this system the amount of intracellular fatty acids or triglycerides can increase.

Decompensation of the thermoregulatory system [18] with decrease of the body core temperature leads to cardiovascular dysfunction resulting in hypotonia and bradycardia including hypoxaemia and hypoxia of the tissue [1]. Also, ischaemic conditions of the organs lead to a disturbance of the intracellular fatty acid mechanism. The cellular fatty acid mechanism is depending on oxygen. Exceeds the oxygen demand the oxygen levels, the mitochondrial function is disturbed and the rate of fatty acid oxidation is reduced. The utilization of free fatty acids for the synthesis of ketone bodies, phospholipids and cholesterol is inhibited. Furthermore, in hypoxic tissue the uptake of lipids is increased caused by hypoxia induced fatty acid transporter translocation, shown in myocytes [19]. In this way, under persistent hypoxic conditions an intracellular accumulation of intracellular lipids occurs, seen as vacuoles in the cytoplasm. This has been observed in myocytes, hepatocytes and in renal and cerebral tissue in cases of fatal hypothermia [3, 8, 9, 11].

Preuß *et al.* investigated fatty degeneration of different organ tissues in cases of fatal hypothermia [10]. They found fatty degeneration of renal tubule epithelium in a study group of 83 cases of fatal hypothermic death in 87% of the cases. In contrary a control group of 25 cases presented signs only in 24% of the cases of fatty degeneration. In the present study we found a significant correlation between cause of death and the appearance of fatty degeneration of renal tissue in 41 cases of fatal hypothermia. Our results fit therefore well to the study of Preuß *et al.* the percentage of fatty degeneration in renal tubule epithelium of both study groups is similar.

In our control group of 45 cases (25 cases in the study of Preuss *et al.* [11]) the number of cases presenting fatty degenerated cells was higher than expected but could be explained by the array of different causes of death included in both control groups.

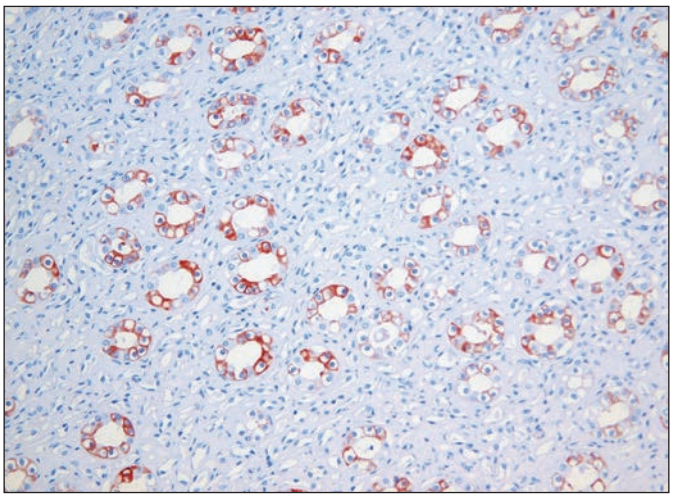

Figure 5. Renal tissue, study group, grade 2, Sudan stain, 200x.

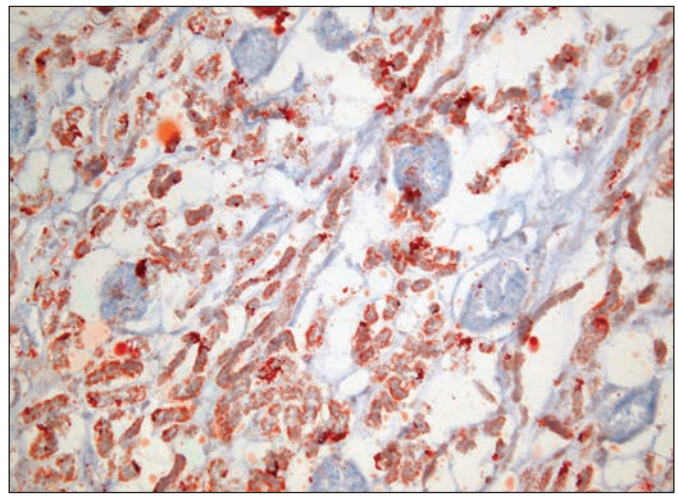

Figure 6. Renal tissue, study group, grade 3, Sudan stain, 200x.

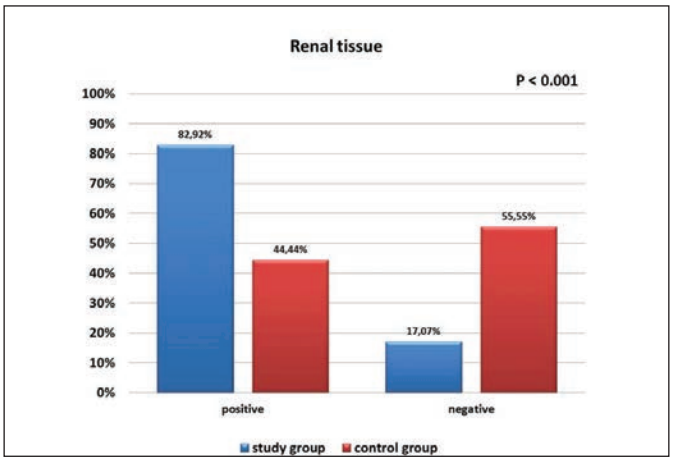

Figure 7. Positive and negative results of Sudan stain of the study and control group.

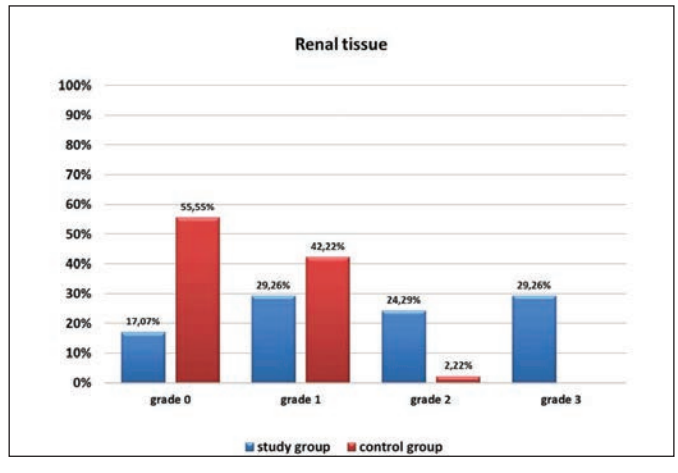

Figure 8. Different graduations of the Sudan stain regarding study and control group.

We showed that fatty degeneration of renal tissue (tubular epithelium as well as glomeruli) is a reliable diagnostic histopathological tool but not a specific marker of hypothermia. Together with circumstantial evidence for hypothermia this finding may support the diagnosis of hypothermia when macroscopic evidence is missing.

CONCLUSIONS

In 2/3 of the cases of fatal hypothermia macroscopical signs of hypothermia (frost erythema, hemorrhagic gastric erosions) are found;

Especially in cases with short terminal episode (quick drop of body core temperature) macroscopical signs of death due to hypothermia may be less severe or even missing;

In such cases histological alterations, such as cellular vacuolation, may support the diagnosis of hypothermia. That has been reported in different organs, the kidney amongst others;

In a case control study the existence of fatty degeneration of renal tubular cells and glomeruli was investigated;

Compared to the control group, fatty degeneration of renal tubular cells and glomeruli was observed more frequently in the study group and furthermore also more intensive;

In cases of circumstantial evidence indicating death due to hypothermia fatty degeneration of renal tubular cells – although unspecific – may support the diagnosis;

A previous study reported a correlation between macroscopic findings in death due to hypothermia and the level of fatty degeneration. This could not be confirmed by our own results;

It should be studied further in prospective investigations if and in what cases of death due to hypothermia ketoacidosis may be a predisposing and concommitting factor for fatty degeneration.

Conflict of interest. The authors declare that there is no conflict of interest.

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