DISTRIBUTION OF HYDROGEN SULPHIDE IN RATS' ORGANS AND ASSOCIATED HISTOLOGICAL CHANGES IN EXPERIMENTAL INTOXICATION

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ABSTRACT: Chemistry-based diagnosis in cases of sudden death due to unnatural causes in cases of anoxemic deaths (e.g. in sewage collecting reservoirs) requires the possability to estimate hydrogen sulphide levels in autopsy material. The usefulness of the analytical techniques spectrophotometric methods and gas chromatography „headspace" techniques with a thermal conductivity detector (TCD) for identification and determination of hydrogen sulphide in biological material were evaluated. Hydrogen sulphide determinations in biological samples taken from rats lethaly intoxicated with gaseous hydrogen sulphide, permitted the selection of the most suitable autopsy material needed for routine toxicological analyses and its histological assessment.

KEY WORDS: Hydrogen sulphide; UV-VIS spectrometry; Gas chromatography.

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Sudden death of persons exposed to extremal conditions of extremely elevated hydrogen sulphide levels require the quantitative estimations of its content in the autopsy material. The problems pertain some cases of sudden death in restricted concentrations of oxygen e.g. in sewage collecting reservoirs, chemical industry or laboratories using hydrogen sulphide, as well as during unconontrolled eruption of natural gas in to the atmosphere [5, 10, 11].

In order to evaluate the effect of hydrogen sulphide and of oxygen deficit in lethal intoxications, chemical diagnosis and histological studies on the autopsy material are indispensable. Analysis of hydrogen sulphide in biological material poses some difficulties related to its volatile nature, rapid biotransformation to sulphates and absence of reliable data as to the choice of the proper autopsy material for the studies.

Toxicological studies on biological material require appropriate analytical techniques and, till now, have been based on UV-VIS spectrophotometry [2, 4, 9] and gas chromatography [6, 7, 8]. In the study performed, accummulation and distribution of hydrogen sulphide were evaluated in selected organs of the rat in the conditions of experimental intoxications and in the course of natural biodegradation of the agent.

In diagnostic studies, the presence of hydrogen sulphide and its level were established using spectrophotometry [2]. An attempt was made to confirm its presence as well as to establish the presence of other volatile inorganic compounds using chromatography. Exponents of hydrogen sulphide toxicity were compared to the histopathological lesions in selected organs of the rats.

MATERIALS AND METHODS

Course of the animals experiments

Intoxication was induced in a chamber (glass exiccator) of approximate volume 3 dm^3. Hydrogen sulphide gas, was generated directly in the exiccator from sodium sulphide and a suitable amount of 10% sulphuric acid. The concentration of hydrogen sulphide, body weight of adult male wistar rats and time of lethal intoxications are listed in Table 1.

The studies pertained toxic action of hydrogen sulphide, studied in two groups of animals (each of 5 rats) exposed to different levels of hydrogen sulphide, sufficient to induce death of the rats in 5 min and in 10–30 min, respectively. Immediately after the death, 2 ml blood was taken and whole individual organs were isolated and placed in water of the same volume (liver, lungs, kidneys) or of the twice lower volume (femoral muscle). The organs were homogenised in a an agate mortar and 2 g of the obtained liquid homogenate were transferred to Conway's chamber. Two ml 10% H_2SO_4 were added, the system was sealed and gently mixed. The microdiffusion process was conducted at 37°C for 90 min using 0.1 mol/l (3 ml) NaOH as the medium which absorbed the released hydrogen sulphide.

The organs (brain, liver, kidneys, lungs, spleen, muscules) for selected histological examinations were fixed in 10% formaldehyde or in Bouin's fixative and stained with hematoxiline and eosine reagents (H + E).

Procedure of analysis

Spectrophotometric methods

The analysis of hydrogen sulphide content in biological material was based on the specific colour reaction in which hydrogen sulphide reacts with N,N-dimethyl-p-phenyldiamine leadding to formation of methylene blue. The phenomenon of hydrogen sulphide diffusion from the acidic medium of standard solutions or a biological material to NaOH solution taking place in the Conway's chamber was employed. Portions of 1 ml of the studied solution were placed in the outer chamber, supplemented with increasing concentrations of Na_2S, which warranted release of hydrogen sulphide in the appropriate range of levels. The standard solutions

were prepared from the aqueous stock solution (0.7059 g $Na_2S \cdot 9\ H_2O$/l, corresponding to 0.1 g H_2S/l).

The standard solutions in the range (4 to 50 mg/10 ml) were prepared in the plasma. The process of microdiffusion was conducted analogously to that in the biological material. After 90 min 2 ml of the absorbing solution (0.1 N NaOH) was taken from the outer part of the chamber and transferred to 2 ml of the reagent, containing 1 ml $FeCl_3$ (200 mg $FeCl_3 \cdot 6H_2O$ in 100 ml 10% HCl and 1 ml N,N-dimethyl-p-phenyldiamine/100 mg in 100 ml 5% HCl). Ten minutes after the mixing of the contents the measurement was performed at l = 670 nm.

Gas chromatographic procedure

Analysis of hydrogen sulphide in parallel with other gases (CO, CO_2, HCN, SO_2)

The presence of hydrogen sulphide in the biological material was confirmed by gas chromatography with the headspace technique.

Qualitative and quantitative analysis of the studied compounds was conducted in the Chrom-5 gas chromatograph, equipped with the dual thermal conductivity detector (TCD). Glass columns of 3.5 m length and 3 mm diameter were used at the catarometer bridge current of 160 mA and hydrogen was a carrier gas (30 ml/min). In analysis of hydrogen sulphide the column was packed with Haye-Sep Q or Porapak Q (80–100 mesh) Alltech Associates (Applied Science) and was activated at 200°C for 5 h at the nitrogen flow rate 20–30 ml/min. Temperature parameters required for the optimum performance were stable and included the injection port and the oven temperatures of 50°C and 40°C respectively. In analysis of carbon monoxide as well as air compounents the column was packed with the molecular sieve 5A (80–100 mesh) and activated according to the producer requirement (Applied Science) qualitative and quantitative analyse were performed using the "headspace" technique based an the analysis of vopour over the solution.

Headspace vial of 4.5 ± 0.1 ml volume, with 1 ml analogously prepared standard solution (Na_2S x 9 H_2O) which corresponded to H_2S levels ranging between 0.2 and 3 mg/10 ml, was charged with 0.5 ml KCN, which corresponded to 1 mg/10 ml HCN (internal standard). After sealing the vial, 0.5 ml 10% H_2SO_4 was injected to it through the septum. The vial was placed at 30°C and 5 min later 0.5 ml of the vapours over the tested solution was taken using the Hamilton Gastight # 1001 syringe. The sample was injected for 10 s to the injection port. In cases of biological material analysis, 1 g tissue homogenate was introduced to the headspace vial, external standard was added and, then, the standard procedure was followed.

RESULTS AND DISCUSSION

Analytical studies have demonstrated the suitability of the spectrophotometric technique for analysis of hydrogen sulphide in the biological material. The calibration range, expressed by the amounts of estimated hydrogen sulphide in individual samples (ranging between 0.4 and 5 mg H_2S) permitted a determination of H_2S levels in rat organs following the intoxication. The Ab = f (c) relation within the studied range was characterised by a high correlation coefficient (0.99) a the favourable value of the coefficient at the relative standard deviation of 1.23% (n = 10 assays). The suitability of the technique for determination of H_2S levels in organs and blood of experimentally intoxicated rats was corroborated. The conditions of intoxication at the variable H_2S levels are characterised in Table I.

TABLE I. CONDITIONS OF EXPERIMENTAL INTOXICATION

Rat no.	Body weight [g]	Generation of H_2S in the exiccator			Duration of intoxication till death [min]
		$Na_2S \cdot 9\ H_2O$ [g]	H_2SO_4 10% [ml]	H_2S [mg/l]	
1	290	1.0	15	47.2	04’30’’
2	301	1.0	15	47.2	05’20’’
3	303	1.0	15	47.2	05’55’’
4	291	1.0	15	47.2	07’30’’
5	286	0.40	8	18.9	03’30’’
6	300	0.25	5	11.8	12’30’’
7	285	0.20	4	9.4	10’20’’
8	287	0.20	4	9.4	11’10’’
9	285	0.10	2	4.7	19’05’’
10	300	0.10	2	4.7	31’01’’

Analysis of hydrogen sulphide levels in organs of lethally intoxicated rats clearly demonstrated variable levels irrespetively of the conditions in which the intoxication developed.

The hydrogen sulphide concentrations detected in individual organs (Table II) confirmed the earlier data [8]. As noted earlier, we have demonstrated peak hydrogen sulphide levels of a decreasing order in kidneys, liver, brain, muscles and lungs. The lowest hydrogen sulphide levels were detremined in the blood of the intoxicated animals. This seems to indicate a restricted value of blood tests in supravital diagnosis, e.g. in clinical toxicology situations. Nevertheless, literature data on the subject point to the possibility of detecting hydrogen sulphide in the blood of intoxicated individuals obtained upon autopsy [2]. However, interpretation of the levels and toxic action should in such cases be cautious due to the possible occurrence of endogenous hydrogen peroxide. Our studies showed that the same blood sample was free of hydrogen sulphide

after 3 months of storage at ± 5°C for three months but contained 4.5 mg/ml hydrogen sulphide after 10 days of storage at 18°C.

TABLE II. THE HYDROGEN SULPHIDE CONCENTRATIONS IN AUTOPSIED RATS TISSUES [mg/g]

Organ/ rat no.	1	2	3	4	5	6	7	8	9	10	$\overline{X}$
Kidneys	12.35	11.55	10.71	10.71	12.24	12.49	10.0	11.29	11.23	9.67	11.22
Liver	8.70	6.52	7.63	6.52	8.48	8.82	6.50	9.15	6.27	5.19	7.37
Brain	5.59	5.91	5.57	5.87	5.44	5.48	6.00	4.60	5.54	4.83	5.48
Muscle	–	2.62	4.16	3.40	–	3.30	–	3.98	4.32	3.22	3.57
Lung	1.91	1.49	1.94	1.81	–	1.76	–	1.85	1.30	1.20	1.65
Blood	The lower limit of calibration curve										

The obtained exponents of the toxic effect of H_2S on living organisms and the differentiated affinity of the compound to individual organs provided indications for respective histological studies. The results are as follows.

No alteration were noted in histological structure of muscles, liver, spleen and brain, relative to that in the control animals. In kidneys of the hydrogen sulphide exposed animals numerous erythrocytes were disclosed in capillaries of renal glomeruli as well as in interlobular vessels (Figure 1). A similar effect was observed in the lungs, more erythrocytes were present in the vessels of interalveolar septa than in the control animals (Figure 2).

Inflammatory infiltrates, exudates or signs of fibrosis were not detected in any of the organs studied.

To corroborate the above results, additional gas chromatography study with the thermal conductance detection (TCD) and the headspace technique were performed. The Haye Sep Q and Porapak Q columns permitted a qualitative analysis of H_2S in parallel with the most frequently encountered inorganic gases (CO, HCN, SO_2, CO_2). The presence of SO_2 in the mixtures in parallel with H_2S restricts their detectability due to their counteracting oxidoreductive properties and formation of non-volatile sulphur. On the other hand, the use of 5A molecular sieve prowed unsuitable in analysis of volatile compounds of acidic oxide (CO_2, SO_2) or acid (HCN) types, including H_2S, due to their selective reactivity with oxides of metals form the molecular structure of the sieve. The 5A sieve may be used for determination of oxygen levels in the athmosphere in which intoxication developed and for analysis of CO (in HbCO). The effects of separation of the discussed inorganic gas mixtures are presented in Figure 3.

The developed chromatographic technique of qualitative and quantitative analysis of H_2S using TCD and headspace technique may be used as the confirming approach. The choice of HCN as an internal standard was based on the similarity of its physicochemical properties, to those of H_2S, including the release from potassium salts in acidic environment. Statistical analysis of reproducibility of the results obtained

Fig. 1. Micrograph of a section of the kidney from a rat exposed to hydrogen sulphide (left) and control (right).

Fig. 2. Micrograph of a section of the lung from a rat exposed to H_2S (left) and control (right).

within the callibration range of 0.2–5 mg/10 ml H_2S has documented a favourable relative standard deviation (5.84%) obtained in n = 10 testings of assays. Comparative appraisal of the two techniques proved that the UV-VIS spectrophotometric technique was 50 times more sensitive as compared to the chromatographic technique with TCD. The results permitted verification of the hitherto collected data on the selection of

Fig. 3. Chromatographic separation of volatile inorganic compounds on Haye Sep Q, A – standard mixture, B – analysis of hydrogen sulphide in autopsy material – purtrefacted liver, 1 – N_2, O_2, 2 – CO, CO_2, 3 – H_2O, 4 – H_2S, 5 – HCN, 6 – SO_2.

appropriate autopsy material and the appropriate methods of hydrogen sulphide analysis in cases suspected of such intoxication.

CONCLUSIONS

1. The UV-VIS spectrophotometric method seems most suitable for routine analysis of biological material (range: 0.1–5.0 mg/l).
2. The microdiffusion technique represents the most effective way of isolating H_2S from biological material, for quantitative assessment of intoxication.
3. In qualitative and quantitative analysis of lethal H_2S intoxication in rats, the most appropriate biological material includes kidneys, liver and brain while muscles and lungs are less suitable because of lower absorbability of hydrogen sulphide.
4. The blood of intoxicated animals exhibits the lowest level of H_2S and, therefore, blood tests are of restricted value for diagnostic purposes in clinical and forensic toxicology.
5. The results permitted verification of the present views and recommendation as to the preservation of appropriate biological material for purposes of chemical diagnosis in H_2S intoxications.
6. The kidneys and lungs of H_2S exposed animals indicated some histological changes, (numerous erythrocytes) relative to their status in the control group.

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