

HYDROGEN SULFIDE: METABOLISM, BIOLOGICAL AND MEDICAL ROLE

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Hydrogen sulfide (H_2S) is a signaling molecule that is actively synthesized in the tissues and is involved in the regulation of vascular tone, neuromodulation, cytoprotection, inflammation and apoptosis. In recent years, new data on animal and human H_2S metabolism and function under the effect of various endogenous and exogenous factors, including drugs were collected. This review is provided to introduce generalized information about the main and alternative H_2S metabolism and regulation, peculiarities of transport, signaling, biological role and participation in pathogenesis. Submitted data describe H_2S content and activity of H_2S -synthesizing enzymes in different organs, H_2S effect on blood coagulation and platelet aggregation based on our research results. The working classification of H_2S metabolism modulators, which are used in biology and medicine, is proposed: 1) agents that increase H_2S content in tissues (inorganic and organic H_2S donors; H_2S -synthesizing enzymes substrates and their derivatives, H_2S -releasing drugs; agents that contain H_2S -synthesizing enzymes cofactors and activators, agents that inhibit H_2S utilization); 2) agents that reduce H_2S content in tissues (specific and nonspecific inhibitors of H_2S -synthesizing enzymes), 3) agents with uncertain impact on H_2S metabolism (some medicines). It was demonstrated that vitamin-microelement and microelement complexes with H_2S -synthesizing enzymes cofactors and activators represent a promising approach for H_2S content correction in tissues.

Key words: hydrogen sulfide, H_2S -signaling, enzymes, regulation, H_2S metabolism modulators, vitamin and microelement complexes.

Hydrogen sulfide (H_2S) is well-known as a toxic gas with obnoxious odour, which is mainly formed in the process of putrefaction of animal and plant proteins. Its role in living organisms was considered for a long time only from the viewpoint of exotoxicant and endotoxicant, meaning the inhibitor of cytochrome *c* oxidase, monoamine oxidase, neurotoxin, and lung irritant. The interest to H_2S significantly increased from the beginning of the 90's of the 20th century because of determination of its connection with regulation of animal and human physiological functions. The results of studies of Kazuho Abe та Hideo Kimura (1996) have triggered more deep research of H_2S biological role. The studies described peculiarities of this metabolite production in rats' brain and identified its first molecular target: N-methyl-D-aspartate receptors (NMDA-receptors) [1].

H_2S is up to date a significant member of gas-transmitter family, including nitrogen monoxide (NO) and carbon monoxide (CO), and is involved in vascular tone regulation, neuromodulation, cytoprotection, inflammation, apoptosis and other processes [2–7]. Despite of the great number of foreign articles describing the role of H_2S in biology and medicine, the Ukrainian works on this issue are scarce. New information has been accumulated recently on metabolism and H_2S functions in organisms under the effect of different endogenous and exogenous factors, including medicines, which we tried to generalize in this review.

H_2S physicochemical properties and membrane transport. H_2S is a short-living molecule with half-life of a few minutes [2]. In aqueous solutions 20-30% of H_2S exist in a non-dissociated form at pH 7.4 and 70-80% in a form of hydrosulfide anion (H_2S

$\leftrightarrow \text{H}^+ + \text{HS}^-$; $\text{p}K_a$ 6.89), partially transforming into sulfide anion (S^{2-}) [3, 8]. H_2S possesses a high lipophilicity and is solved in lipid membranes two times easier (partition coefficient – 2.0 ± 0.6), than in water [8]. H_2S is characterized by high permeability coefficient of lipid membrane ($P_m = 3 \text{ cm}\cdot\text{s}^{-1}$) and easily diffuses throughout cell membranes [8]. In conditions of organism at pH 7.4, the transmembrane diffusion of H_2S proceeds slower ($P_{m7.4} = 0.85 \text{ cm}\cdot\text{s}^{-1}$), that leads to local accumulation of this molecule near a cell-producent. It has been determined throughout 3D-mathematic modelling that the sphere of H_2S biological action is determined by that distance, at which metabolite's concentration is no less than 10% of its concentration in the place of synthesis, and is spread over 200 neighbouring cells per 1 sec on the average [8]. Thus, H_2S acts as paracrine signaling molecule, but the distant effect is not excluded, because this metabolite is a part of blood plasma and may be transported by erythrocytes. It is determined that H_2S transport by human erythrocytes proceeds in 4 stages: 1) diffusion of H_2S throughout cell membranes or gas channels; 2) extracellular deprotonation of H_2S into HS^- ; 3) arrival of HS^- into erythrocyte throughout anion transporter – protein AE1 at the exchange for Cl^- ; 4) intracellular HS^- protonation to H_2S [9]. The membrane transport of H_2S can also occur throughout aquaporins – water channels [10].

Biosynthesis of H_2S . Major sources of endogenous H_2S in tissues are sulfur-containing amino acids – L-cysteine and L-homocysteine, which metabolized in reactions of transsulfuration and transamination with participation of pyridoxal 5'-phosphate dependent enzymes of cystathionine γ -lyase (CSE, EC 4.4.1.1), cystathionine β -synthase (CBS, EC 4.2.1.22); 2) and cysteine aminotransferase (CAT, EC 2.6.1.3) (Table 1). Key reactions providing production of H_2S in tissues of animals and humans are as follows: 1) desulfuration of L-cysteine to pyruvate (α , β -elimination) by CSE; 2) condensation of L-homocysteine with L-cysteine (β -replacement) and desulfuration of L-cysteine to L-serine (β -elimination) by CBS; 3) transamination of L-cysteine with α -ketoglutarate by CAT with production of 3-mercaptopyruvate, out of which H_2S is further emitted with participation of 3-mercaptopyruvate sulfur transferase (3-MST, EC 2.8.1.2). Co-factors of 3-MST in this reaction may be glutathione, thioredoxin and dihydrolipoic acid [11, 12].

Other ways of enzymatic H_2S synthesis were detected recently, physiologic meaning of which is not finally clarified (Table 2) [12–15]. Alternative

sources of H_2S are pyridoxal 5'-phosphate dependent reactions: 1) desulfuration of L-cystine to L-thiocysteine with next H_2S releasing (α , β -elimination) by CSE; 2) desulfuration of L-cysteine to L-serine (β -elimination) by CBS; 3) condensation of two molecules of L-homocysteine (γ -replacement) to L-homolanthionine by CSE; 4) desulfuration of L-homocysteine to L-homoserine by CSE; 5) condensation of two molecules of L-cysteine (β -replacement) to L-lanthionine with the participation of CSE or CBS; and also pyridoxal 5'-phosphate-independent reactions: 6) D-cysteine oxidation to 3-mercaptopyruvate by D-amino acid oxidase (DAAO, EC 1.4.3.3); 7) thiosulfate-anion reduction by thiosulfate-dithiol sulfurtransferase (TST, EC 2.8.1.5).

Kinetic parameters of key and alternative pyridoxal 5'-phosphate dependent reactions of H_2S synthesis, which have been studied *in vitro* on the example of human recombinant enzymes CSE and CBS, are significantly distinct. Investigations of Singh (2009) have shown that 96% of H_2S is produced in the condensation of L-cysteine with L-homocysteine with the participation of CBS with V_{\max} 18.7 U/mg and K_m 3.2 mM. Alternative reactions of H_2S synthesis with the participation of CBS have such kinetic parameters: 1) desulfuration of L-cysteine into pyruvate with V_{\max} 0.82 U/mg protein and K_m 27.3 mM; 2) lanthionine synthesis from L-cysteine with V_{\max} 0.77 U/mg protein and K_m 45,6 mM [13].

Chiku et al. have shown (2009) that among reactions of H_2S synthesis with the participation of CSE only cysteine desulfuration (α , β -elimination) can play the main role because its kinetic parameters are V_{\max} 0.6 U/mg protein and K_m 1.7 mM. It appeared that K_m of alternative CSE-dependent reactions of H_2S synthesis associated with lanthionine, homolanthionine and homoserine creation are significantly higher: 33; 5.9; 2.7 mM at V_{\max} 1.2; 6.6; 1.2 U/mg, respectively [14]. Kinetic parameters of H_2S synthesis from cystine are not determined, because under conditions close to physiological ones, the reaction does not proceed.

3-MST is pyridoxal 5'-phosphate-independent enzyme, which is functionally connected with CAT and, possibly, with TST. As opposed to CSE and CBS, for which pH 8.5-9.0 is optimal, 3-MST effectively synthesizes H_2S from 3-mercaptopyruvate at pH 7.4. This enzyme provides creation of persulfides, from which H_2S can release under interaction with thioles (glutathione, dihydrolipoic acid, thioredoxin) [16, 17]. Also, 3-MST may turn sulfite-

Table 1. Key enzymatic reactions of H₂S creation in tissues of animals and humans

Enzyme	Scheme of reaction	Ref.
Cystathionine γ-lyase (EC 4.4.1.1)	$ \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{L-cysteine} \end{array} + \text{H}_2\text{O} \longrightarrow \begin{array}{c} \text{COOH} \\ \\ \text{C}=\text{O} \\ \\ \text{CH}_3 \\ \text{pyruvate} \end{array} + \text{NH}_3 + \text{H}_2\text{S} $	[13, 14]
Cystathionine β-synthase (EC 4.2.1.22)	$ \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{L-cysteine} \end{array} + \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{L-homocysteine} \end{array} \longrightarrow \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}_2\text{C}-\text{S}-\text{CH}_2-\text{CH}_2 \\ \text{L-cystathionine} \end{array} + \text{H}_2\text{S} $	[13, 14]
Cysteine aminotransferase (EC 2.6.1.3)	$ \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{L-cysteine} \end{array} + \begin{array}{c} \text{COOH} \\ \\ \text{C}=\text{O} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{COOH} \\ \alpha\text{-ketoglutarate} \end{array} \longrightarrow \begin{array}{c} \text{COOH} \\ \\ \text{C}=\text{O} \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{3-mercaptopyruvate} \end{array} + \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{COOH} \\ \text{L-glutamate} \end{array} $	[13, 14]
3-mercaptopyruvate sulfur transferase (EC 2.8.1.2)	$ \begin{array}{c} \text{COOH} \\ \\ \text{C}=\text{O} \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{3-mercaptopyruvate} \end{array} \xrightarrow[\text{- pyruvate}]{+\text{R-SH}} \text{R-S-SH} \xrightarrow[\text{+ R-S-S-R}]{+\text{R-SH}} \text{H}_2\text{S} $ <p style="text-align: center;">persulfide</p> $ \begin{array}{c} \text{COOH} \\ \\ \text{C}=\text{O} \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{3-mercaptopyruvate} \end{array} \xrightarrow[\text{- pyruvate}]{+\text{SO}_3^{2-}} \text{S}_2\text{O}_3^{2-} \dashrightarrow \text{H}_2\text{S} $	[11, 12]

anion into thiosulfite-anion, which is further reduced to H₂S with the participation of TST [11, 12, 18].

The question is how H₂S general production may provide reactions, which necessitate significantly high substrate concentrations (L-cysteine and L-homocysteine). As it is known, under organism conditions, the pull of free sulfur-containing amino acids is significantly lower, than that of their bound and disulfide forms. The content of general homocysteine in human blood plasma does not exceed

15 μM, that of cysteine is 300 μM, but the content of their free (thiol) forms is about 0.13-0.17 and 24-27 μM, respectively [19]. Their quantity under pathology conditions may grow significantly: under homozygous deficit of CBS the level of general homocysteine in the blood plasma increases up to 100-500 μM and above [20], the content of free cysteine may 60 times exceed the norm under chronic kidney failure. Part of free cysteine in blood plasma of pre-hemodialysis patients was 40.9% compared

Table 2. Alternative enzymatic reactions of H₂S creation in tissues of animals and humans

Enzyme	Scheme of reaction	Ref.
Cystathionine γ-lyase (EC 4.4.1.1)	$ \begin{array}{c} \text{COOH} \quad \text{COOH} \\ \quad \quad \\ \text{H}_2\text{N}-\text{C}-\text{H} \quad \text{H}_2\text{N}-\text{C}-\text{H} \\ \quad \quad \\ \text{H}_2\text{C}-\text{S}-\text{S}-\text{CH}_2 \\ \text{L-cystine} \end{array} + \text{H}_2\text{O} \xrightarrow{-\text{NH}_3 - \text{pyruvate}} \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}_2\text{C}-\text{S}-\text{S}-\text{H} \\ \text{L-thiocysteine} \end{array} \xrightarrow[-\text{R-S-S-cysteine}]{+\text{RSH}} \text{H}_2\text{S} $	[13,14]
	$ \begin{array}{c} \text{COOH} \\ \\ 2 \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{L-cysteine} \end{array} \longrightarrow \begin{array}{c} \text{COOH} \quad \text{COOH} \\ \quad \quad \\ \text{H}_2\text{N}-\text{CH} \quad \text{H}_2\text{N}-\text{CH} \\ \quad \quad \\ \text{H}_2\text{C}-\text{S}-\text{CH}_2 \\ \text{L-lanthionine} \end{array} + \text{H}_2\text{S} $	[13,14]
	$ \begin{array}{c} \text{COOH} \\ \\ 2 \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{L-homocysteine} \end{array} \longrightarrow \begin{array}{c} \text{COOH} \quad \text{COOH} \\ \quad \quad \\ \text{H}_2\text{N}-\text{CH} \quad \text{H}_2\text{N}-\text{CH} \\ \quad \quad \\ \text{CH}_2 \quad \quad \text{CH}_2 \\ \quad \quad \\ \text{H}_2\text{C}-\text{S}-\text{CH}_2 \\ \text{L-homolanthionine} \end{array} + \text{H}_2\text{S} $	[13,14]
	$ \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{L-homocysteine} \end{array} + \text{H}_2\text{O} \longrightarrow \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{H}_2\text{C}-\text{OH} \\ \text{L-homoserine} \end{array} + \text{H}_2\text{S} $	[13,14]
Cystathionine β-synthase (EC 4.2.1.22)	$ \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{L-cysteine} \end{array} + \text{H}_2\text{O} \longrightarrow \begin{array}{c} \text{COOH} \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}_2\text{C}-\text{OH} \\ \text{L-serine} \end{array} + \text{H}_2\text{S} $	[13,14]
	$ \begin{array}{c} \text{COOH} \\ \\ 2 \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{L-cysteine} \end{array} \longrightarrow \begin{array}{c} \text{COOH} \quad \text{COOH} \\ \quad \quad \\ \text{H}_2\text{N}-\text{CH} \quad \text{H}_2\text{N}-\text{CH} \\ \quad \quad \\ \text{H}_2\text{C}-\text{S}-\text{CH}_2 \\ \text{L-lanthionine} \end{array} + \text{H}_2\text{S} $	[13,14]
D-amino acid oxidase (EC 1.4.3.3)	$ \begin{array}{c} \text{COOH} \\ \\ \text{H}-\text{C}-\text{NH}_2 \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{D-cysteine} \end{array} \xrightarrow[-\text{NH}_3 - \text{H}_2\text{O}_2]{+\text{H}_2\text{O} + \text{O}_2} \begin{array}{c} \text{COOH} \\ \\ \text{C}=\text{O} \\ \\ \text{H}_2\text{C}-\text{SH} \\ \text{3-mercaptopyruvate} \end{array} \xrightarrow{\text{---}} \text{H}_2\text{S} $	[12]
Thiosulfate- dithiol sulfurtrans- ferase (EC 2.8.1.5)	$ \text{S}_2\text{O}_3^{2-} + 2\text{R-SH} \rightarrow \text{SO}_3^{2-} + \text{R-S-S-R} + \text{H}_2\text{S} $	[22,29]

to 1.6% in healthy persons [21]. Obviously, the role of alternative reactions of H₂S synthesis at ordinary concentrations of sulfur-containing amino acids in organism is insignificant, but it may increase under hyperhomocysteinemia or hypercysteinemia, which are often combined [19].

The role of pyridoxal phosphate-independent ways of H₂S creation (from D-cysteine and thiosulfate) in animal and human organism has not been studied completely. Studies of Shibuya and Kimura (2013) prove that optimal conditions for H₂S synthesis from D-cysteine exist in organism (pH 7.4). However, this amino acid is not formed in the cells and may arrive only from exogenous sources [12]. The role of thiosulfate-anion and TST in creation of H₂S in tissues is the least studied. We have no clear data as of today regarding the content of thiosulfate-anion in organs and tissues, thus it is difficult to estimate the importance of this enzyme. Allowing for the concentration of thiosulfate-anion in blood plasma and urine of humans (1.13 ± 0.11 mg/dl and 0.28 ± 0.02 mg/dl, respectively) [22], the above-mentioned way of H₂S formation in tissues is possible.

Catabolism and deposition of H₂S. H₂S catabolism may be performed by enzymatic and non-enzymatic ways (Table 3). In mitochondria HS⁻ is oxidated to thiosulfate-anion and sulfite-anion by sulfide quinone oxido-reductase system (SQR). Then sulfite is converted to sulfate by sulfite oxidase (EC 1.8.3.1). SQR consists of three enzymes: thiosulfate:cyanide sulfur-transferase (rhodanese,

EC 2.8.1.1), sulfur dioxygenase (EC 1.13.11.18) and sulfide:quinone reductase (EC 1.8.5.4) [23]. H₂S may spontaneously react with mitochondrial hemoproteins – cytochrome oxidase and cytochrome *c* with creation of sulfane sulfur (S⁰) and reactive thiol radical (HS[•]) [23, 24].

In cytosol H₂S methylates to methanethiol and dimethyl sulfide with participation of thiol S-methyltransferase (EC 2.1.1.9). Utilization of H₂S in erythrocytes proceeds in non-enzymatic way through formation of sulfhemoglobin [3, 2, 23].

H₂S catabolism by SQR and its direct reaction with thiols results in formation of unstable persulfides (R-S-S^{*}-H, thioaurine, thiocysteine) which contain active sulfane sulfur (S⁰) [23]. H₂S is deposited in sulfane sulfur form in different tissues (brain, heart, liver, kidney) and can be released on demand. 3-MST and CAT provide H₂S deposition in polysulfides [25, 26]. The quantity of polysulfides in cells with 3-MST and CAT expression is twice as much in comparison with cells without these enzymes [26].

H₂S participates in formation of nitrosothiols (RSNO) known as depot of NO in cells [27, 28]. HS⁻ or HS[•] may interact with active forms of nitrogen (NO[•], ONOO⁻) with creation of the smallest nitrosothiol – thionitrous acid (HSNO) [27]. HSNO, however, reacts with thiols with formation of nitrosothiols and H₂S [27].

Features of H₂S tissue metabolism and its regulation. Organs and tissues are distinguished by the ability to produce H₂S-synthesizing enzymes

Table 3. Utilization and deponation of H₂S in tissues

Enzyme	Scheme of reaction	Ref.
Sulfide:quinone reductase (EC 1.8.5.4)	$H_2S + R-SH + \text{quinone} \rightarrow R-S-S^*-H + \text{hydroquinone}$	[24]
Sulfur dioxygenase (EC 1.13.11.18)	$R-S-S^*-H + O_2 + H_2O \rightarrow R-SH + SO_3^{2-} + 2H^+$	[23]
Thiosulfate:cyanide sulfur-transferase (EC 2.8.1.1)	$2HS^- + 2O_2 \rightarrow S_2O_3^{2-} + H_2O$ $S_2O_3^{2-} + CN^- \rightarrow SCN^- + SO_3^{2-}$	[23]
Sulfite oxidase (EC 1.8.3.1)	$SO_3^{2-} + Fe^{3+} (\text{cytochrome } c) \rightarrow SO_4^{2-} + Fe^{2+} (\text{cytochrome } c)$	[3, 23]
Thiol S-methyltransferase (EC 2.1.1.9)	$H_2S \rightarrow CH_3-SH \rightarrow CH_3-S-CH_3$	[23]
Non-enzymatic way	$H_2S + \text{methemoglobin} \rightarrow \text{sulfhemoglobin}$ $HS^- + Fe^{3+} (\text{cytochrome } c) \rightarrow Fe^{2+} (\text{cytochrome } c) + HS^•$	[23]
Non-enzymatic way	$HS^• + NO^• \rightarrow HSNO$ $HSNO + RSH \leftrightarrow RSNO + H_2S$	[27, 28]

and ability to produce H₂S (Table 4). In animal and human organism H₂S is formed in the liver where all H₂S-synthesizing enzymes (CBS, CSE, CAT, 3MST) are expressed [5, 29–31]. Also H₂S is intensively formed in kidneys. CSE and CBS are expressed in renal cortex and medulla, proximal tubules, interstitial tissue [32], but 3-MST is expressed in glomerules [33].

CBS is considered the major producer of H₂S in the central nerve system; it is expressed in the hippocampus, cerebellum, cortex and brain stem [5, 15, 34, 35]. Creation and deposition of H₂S as polysulfide in brain are provided by CAT and 3-MST, which are expressed in the cortex, cerebellum and retina [25]. CBS is localized in astrocytes, and 3-MST – in neurons [17]. Contribution of CSE to production of H₂S in brain is the least, however, this way is considered important in support of redox homeostasis of the brain [36]. CSE activity in the mice brain is about 1% of its activity in the liver. CSE is present in neurons of human brains and its activity is several times higher than that in mice [36].

In blood vessels H₂S is synthesized with participation of CSE which is mainly expressed in smooth myocytes, and with CAT/3-MST, which are expressed in endothelium. The expression of these enzymes has been found in the aorta, lung artery, mesenteric and kidney arteries [3, 5, 11, 17]. CAT/3MST and CSE are expressed in the myocardium [37–39], but CAT/3-MST, CSE and CBS are expressed in skeletal muscles [40]. Considerably high ability of

H₂S creation was detected in the myometrium of rats and humans, in which CBS and CSE are expressed [41, 42]. The expression of CSE and CBS is detected in the intestine, stomach, β-cells of pancreas [43–45], adipocytes [46], lungs [47, 48].

H₂S synthesis regulation. CBS and CSE cells are localized in the cytozole, while 3-MST and CAT – in the mitochondria and cytozole (division of their fractions depends on tissue) [17, 26, 49].

Human and rat CBS is homotetramer and consists of 4 equal subunits with molecular weight of 63 kDa. Each subunit is bound to the cofactors pyridoxal 5'-phosphate, heme and S-adenosylmethionine [5]. S-adenosylmethionine is an allosteric activator of CBS. That is why CBS-dependent H₂S production may increase, if S-adenosylmethionine concentration is elevating in cells [1]. CBS activity is inhibited by CO, which binds with high affinity with heme (K_i 5.6 μM), and NO – only in high supraphysiological concentrations (K_i 360 μM) [50]. One more mechanism of impact on CBS activity, which proves possible participation of enzyme in epigenetic regulation and gene expression, was detected recently. C-terminal fragment of CBS contains a tandem of two domains, which undergoes SUMO-modification (SUMO – small ubiquitin-like modifier) of lysine in position 211 that causes a decrease of enzyme's activity. CBS sumoylation is inhibited by cystathionine [51]. Biological importance of SUMO-modification of CBS remains unidentified so far. Hypothetically, such form of CBS translocation into nucleus with

Table 4. Main H₂S-synthesizing enzymes expression in laboratory animals and human tissues

Organ, tissue	H ₂ S-synthesizing enzymes	Ref.
Liver	CSE ^{1,2,3} , CBS ^{1,2,3} , CAT ¹ , 3-MST ¹	[5, 29–31]
Kidneys	CSE ^{1,2,3} , CBS ^{1,2,3} , CAT ¹ , 3-MST ¹	[5, 29, 30, 32, 33, 38]
Brain	CBS ^{1,2,3} , 3-MST ¹ , CSE ^{1,2,3}	[5, 17, 25, 35, 36]
Myocardium	CSE ^{1,2} , 3-MST ¹	[37–39]
Aorta, endothelium	CAT ¹ , 3-MST ¹ , CSE ²	[5, 11]
Aorta, smooth myocytes	CSE ¹	[52, 101]
Myometrium, placenta	CBS ¹ , CSE ¹	[41, 42]
Skeletal muscles	CSE ¹ , CBS ¹ , 3-MST ¹	[40]
Adipocytes	CSE ¹	[46]
Stomach, intestine	CBS ^{1,2} , CSE ¹²	[43, 78]
Pancreas, β-cells	CBS ² , CSE ²	[45, 44]
Lungs	CSE ^{1,3}	[47, 48]

Notation. Enzyme expression in ¹rat (*Rattus norvegicus*), ²mouse (*Mus musculus*) and ³human tissues

further desumoylation and renovation of ability to H₂S synthesis can take place [51].

H₂S formation by CSE is activated under the action of calmodulin in the presence of 1-2 mM Ca²⁺ [52]. However, basic concentration of Ca²⁺ in cells is around 100 nM, that is why the role of Ca²⁺/calmodulin in regulation of CSE-dependent H₂S production necessitates further study [6]. Under *in vitro* conditions the ability of recombinant human CSE to sumoylation is established, and this process is not blocked by cystathionine [51]. Whether SUMO-modification of CSE under conditions of organism exists and what kind of role this process plays has not been determined yet. Under hypoxia when concentration of cytosolic Ca²⁺ increases, CSE translocation from cytosole to mitochondrion may happen, the process is accompanied by the increase of mitochondrial H₂S production and ATP synthesis [26].

In contrast to CSE, H₂S formation with participation of CAT/3-MST is dose-dependently inhibited by Ca²⁺ (with full blocking in the presence of 2.9 μM Ca²⁺) and does not depend on calmodulin [17]. CAT activity decreases with L-aspartate increase in the medium [49]. 3-MST-dependent H₂S production increases with the increase of thioredoxin and dihydrolipoic acid content in cells [17].

Concentration of endogenous H₂S. H₂S content in blood plasma of animals (rats) and humans is around 50-80 μM [2, 3, 53]. H₂S presence is more considerable in tissues, in particular, the content of this metabolite in the animal brain is around 50-160 μM [17, 2]. According to other data, H₂S content in the brain, myocardium and kidneys of rats is 2.6; 11.4 and 6.7 μg/g of tissue, respectively [54]. We have to mention that measurement of H₂S content in tissues in the majority of investigations is performed by colorimetric method (in compliance with the reaction with N,N-dimethyl-p-phenylenediamine in the presence of FeCl₃), which needs highly acidic

medium and does not exclude H₂S release from the tissue depot. Presumably, intracellular free H₂S concentration is much more less, because pH is around 7-8 in the mitochondria. Free H₂S concentration determined by the method of gas chromatography, was 0.12 μmol/kg of protein (14 nM) in the rat brain, and concentration of acid-labile sulfur was 916 μmol/kg of protein [55].

According to the results of our own studies (Table 5), general H₂S content and specific activity of H₂S-synthesizing enzymes in rats' tissues are on the highest level in the liver and kidneys, slightly lower in myocardium, brain and aorta. Determination of H₂S content and production in tissues was performed as it has been described [22, 56-58].

H₂S biological role and molecular targets. H₂S in organism plays a role of signaling molecule, gasotransmitter; no specific receptors have been found for it. Different ion channels, receptors, enzymes and proteins, regulating numerous biochemical and physiological processes, play a role of H₂S molecular targets (Table 6).

A key mechanism of H₂S-signaling is S-sulfhydration of proteins, post-translation modification with conversion of -SH groups into -SSH, which significantly increases reactivity of cysteine residues and increases functional activity of molecular targets as well [6, 17, 59]. H₂S also reduces disulfide bonds of cystine residues with -SH groups releasing [23]. Redox-modification of proteins with participation of H₂S may be of independent regulatory importance as well as serve as a preparatory stage for S-sulfhydration and other kinds of post-translational S-modification (S-nitrosylation, S-homocysteinylation, S-glutathionylation). For example, activation of NMDA-receptors with participation of H₂S proceeds in two stages: 1) NMDA-receptors become active under reduction of their disulfide form into thiol form, 2) transition of thiol form into persulfide form

Table 5. H₂S content and activity of H₂S-synthesizing enzymes in rats' organs ($M \pm m$, $n = 10$)

Organ, tissue	H ₂ S, nmol/mg protein	Enzyme activity, nmol H ₂ S/min·mg protein			
		CSE	CBS	CAT/3-MST	TST
Liver	3.85 ± 0.21	3.09 ± 0.15	2.75 ± 0.18	2.47 ± 0.10	13.0 ± 0.55
Kidneys	3.27 ± 0.16	1.65 ± 0.11	2.17 ± 0.14	2.49 ± 0.18	3.29 ± 0.14
Brain	1.52 ± 0.08	0.27 ± 0.03	0.57 ± 0.03	0.13 ± 0.03	1.25 ± 0.08
Myocardium	2.37 ± 0.10	0.23 ± 0.03	0	0.52 ± 0.07	1.20 ± 0.06
Aorta	1.28 ± 0.06	0.67 ± 0.04	0	0.59 ± 0.05	1.98 ± 0.08

Table 6. H₂S-signaling: mechanism, targets, biological processes

Mechanisms	Molecular targets	Target cells	Biological processes
<p>1. <i>Modification of proteins:</i> - S-sulfhydration $R-SH \leftrightarrow R-S-SH$ - Redox-modification $R-S-S-R \leftrightarrow 2R-SH$</p> <p>2. <i>Modification of prosthetic groups (heme, Cu)</i></p> <p>3. <i>Interaction with NO, participation in S-nitrosylation of protein</i> $HS^{\cdot} + NO^{\cdot} \rightarrow HSNO \rightarrow RSNO$</p> <p>4. <i>Interaction with ROS, generation of HS[·], S₂O₃²⁻, SO₃²⁻, SO₄²⁻</i></p> <p>5. <i>Interaction with electrophile lipid derivatives (4-hydroxynonenal and others)</i></p>	<p><i>Ion channels:</i> - K⁺-ATP-channels - K⁺ (Ca²⁺)-channels - T- or L-type of Ca²⁺-channels - TRP- channels (TRPV₁, TRPA₁) - Cl⁻-channels</p> <p><i>Receptors:</i> NMDA receptors</p> <p><i>Signaling enzymes, transcription factors:</i> adenylate cyclase protein kinase A protein kinase C protein kinase B (Akt) phosphoinositide 3-kinase (PI3K) phosphodiesterase MAPK, ERK NF-κβ, Nrf2, HIF-1α</p> <p><i>Others:</i> glyceraldehyde-3-phosphate dehydrogenase glutamate-cysteine ligase thioredoxin reductase cytochrome c oxidase cytochrome c monoamine oxidase actin, β-tubulin cystine/glutamate antiporters excitatory amino acid transporters</p>	neurons astrocytes smooth myocytes cardiomyocytes skeletal myocytes pancreatic β-cells endothelium hepatocytes platelets leukocytes renal tubular epithelium gastrointestinal epithelium	neurotransmission nociception vascular tone regulation myocardial contractility cytoprotection inflammation apoptosis angiogenesis platelet aggregation insulin secretion cysteine transport into cells glutathione synthesis tissue respiration aging circadian rhythm gastroduodenal motility

(S-sulfhydration) with a further, more considerable increase of NMDA-receptors activity [17].

Mustafa et al. (2009) obtained direct proofs that S-sulfhydration raises the activity of ATP-sensitive potassium channels (-SSH modification of Kir6,1 subunit), glyceraldehyde-3-phosphate dehydrogenase (-SSH modification Cys150) and increases actin ability to polymerization [59]. S-sulfhydration increases neuroprotective activity of ubiquitin E3 ligase [15]. H₂S impact over other molecular targets, calcium channels of different types, TRP (transient

receptor potential) channels (TRPV₁, TRPA₁), protein kinases, factors of transcription, is going on presumably through S-sulfhydration/ desulfhydration. This is proved by the results of numerous studies *in vivo* and *in vitro* with usage of H₂S donors (NaHS), inhibitors of H₂S-synthesizing enzymes, knock-out of CSE and CBS genes in animals.

H₂S together with NO participates in S-nitrosylation of proteins and low-molecular thiols [28, 27]. S-nitrosylation, in contrast to S-sulfhydration, decreases cysteine's ability to reaction and leads

to further lost of activity of molecular targets. [6, 17, 59]. For example, glyceraldehyde-3-phosphate dehydrogenase is inactivated during S-nitrosylation Cys150 [59]. H₂S, in its turn, may express NO, activating guanylate cyclase and its own signaling ways, from nitrosothiols [27, 28].

H₂S also acts through interaction with prosthetic groups of metalloproteins, reactive oxygen species (ROS), low-molecular electrophilic derivatives. H₂S in high concentrations blocks the activity of cytochrome *c* oxidase, reducing by electrons heme *aa3*, *CuB* and cytochrome *c* [23]. H₂S may reduce Fe³⁺ into Fe²⁺ of methemoglobin with its conversion into a form able to bind and transport oxygen [23]. Under H₂S interaction with ROS, generation of thiyl radical and thiosulfate anion which is transformed into oxygen-containing sulfur derivatives (sulfites and sulfates) is going on. It is not excluded that this way also mediates H₂S signaling, because SO₂ participates in regulation of vascular tonus and contraction of the myocardium [60]. It is proved that Na₂SO₃/NaHSO₃ causes dose-dependent relaxation of isolated rings of rats' aorta *in vitro* [61]. As a nucleophile, H₂S easily interacts with electrophile lipid derivatives, including 4-hydroxynonenal, which is a strong modulator of oxidative stress, cell proliferation, apoptosis [62].

Biological effect of H₂S may be significantly distinct depending on tissue localization of molecular target. H₂S impact on ion channels, for example, is going on in such directions [7, 15, 26, 63, 64]: 1) activation of ATP-K⁺-channels of smooth myocytes of blood vessels, that is associated with vasodilation, lowering of arterial pressure, cardioprotection under ischemia/reperfusion, inhibition of insulin secretion by the β-cells, anti-inflammatory, antinociceptive and anti-apoptosis effects; 2) inhibition of Ca²⁺-channels of L-type in cardiomyocytes (with Ca²⁺ level decrease) and their activation in neurons (with an increase of Ca²⁺ level); inhibition of large K⁺(Ca²⁺)-channels in carotide sinuses and their activation in pituitary cells; 3) activation of Ca²⁺-channels of T-type and TRPV₁ channels, that is associated with H₂S-induced hyperalgesia and pronociceptive effect; activation of TRPA₁-channels with transportation of Ca²⁺ into astrocytes; 4) H₂S-induced contraction of out-vascular smooth muscles and increase Cl⁻ secretion in gastrointestinal tract are connected with activation of TRPV₁ and TRPA₁ channels; 5) Cl⁻ channels activation and prevention from neurons oxytosis.

Year after year more and more mechanisms of H₂S-signaling are identified, where kinases, transcription factors, growth factors and other regulator molecules are involved. H₂S action may be mediated through:

1) modulation of adenylate cyclase, phosphodiesterase activity and cAMP content in cells;

H₂S causes inhibition of adenylate cyclase and decrease of cAMP content in neurons of morphine-dependent mice [65]; phosphodiesterase activity increase in juxtglomerular cells of rats' kidneys with renovascular hypertension [66]; cAMP level and activity of protein kinase A increase but cAMP-dependent phosphodiesterase activity decrease in isolated mitochondria of rats' hepatocytes [67]; cAMP synthesis increase in culture of neurons and glial cells [68].

2) modulation of protein kinases C, PI3K, B (Akt), MAPK, ERK activity;

H₂S induces an increase of activity and expression of protein kinase C [69]; phosphoinositide 3-kinase (PI3K), protein kinaseB (p-Akt), kinase-3β glycogen synthetase (GSK-3β) and protein Bcl-2 [70]; inhibits expression of mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases (ERK) [4, 71], that decreases inflammatory response and disorder of cells under action of different factors.

3) modulation of activity of transcription factors Nrf2, NF-κB, HIF-1α;

H₂S stimulates translocation in the nucleus of Nrf2 – nuclear factor (erythroid-derived nuclear factor of transcription), that activates antioxidant response pathway and up-regulates thioredoxin reductase, glutathione S-transferase, thioredoxin-1 expression [23] and glutathione synthesis [72]; inhibits NF-κB expression (nuclear factor kappaB) and down-regulates proinflammatory cytokines expression [73, 74]; up-regulates expression of HIF-1α (hypoxia-inducible factor-1) and vascular endothelial growth factor (VEGF), which stimulates angiogenesis [23].

4) modulation of NO and CO synthesis and their signaling pathways;

H₂S up-regulates eNOS expression and increases endothelial NO production [23]; decreases iNOS production and nitrosative stress [75]; up-regulates heme oxygenase-1 expression and CO production in cardiomyocytes and other cells [75, 76].

5) impact on aging and circadian rhythm genes (*SIRT1*, *Klotho*) expression, through which formation of age-associated changes in tissues is mediated [77].

Workers of Blood Circulation Physiology Department of the O. O. Bohomolets Institute of Physiology of NAS of Ukraine (Kyiv) under leadership of V. F. Sahach, Corresponding Member of NAS of Ukraine, doctor of medical science, assessed that H₂S is involved into opening of mitochondrial permeability transition pore, which is a key player in development of apoptosis and necrosis, and this effect is realized through K⁺-ATP-channels modulation [102–104].

Direction of H₂S action depends on its content in tissues. At low (physiological) concentrations H₂S shows its vasodilating, cytoprotector, antioxidant, anti-inflammatory and anti-apoptotic effects. It also increases sensibility of NMDA-receptors of neurons to glutamate, stimulates Ca²⁺ transportation to astrocytes and increases synaptic activity. H₂S activates cystine-glutamate antiporters, stimulates transportation of cysteine into mitochondria, and increases activity of γ -glutamylcysteine synthetase (glutamate-cysteine ligase, EC 6.3.2.2) and glutathione synthesis in neurons and other cells [4, 17, 23, 26, 68]. H₂S in supraphysiological and toxic concentration induces mitochondrial dysfunction, blocks tissue respiration and oxidative phosphorylation, increases vascular tonus, causes hyperalgesia, promotes inflammation and apoptosis, inhibits synaptic transmission. H₂S enables biogen amins effects (γ -aminobutyric acid, glutamate, serotonin, dopamine, epinephrine and norepinephrine) and acetylcholine by up-regulation of their receptors expression and inhibition of monoamine oxidase and acetylcholine esterase activity [17, 68]. Low H₂S concentrations increase basal tension, smooth muscle contractions and motility of the gastric antrum, but high H₂S concentrations, on the contrary, decrease all the above-mentioned functions [78]. H₂S in concentration of 0.1–1 μ M increased electron transport and ATP content, and being in a concentration of 3–30 μ M it inhibited cytochrome *c* oxidase and oxidative phosphorylation in mitochondria of isolated hepatocytes [79].

Different pathological conditions are associated with disorder of H₂S content in tissues. A decrease of basic H₂S content in blood plasma is noticed in patients with arterial hypertension, ischaemic heart disease, deep venous thrombosis, Alzheimer's disease, hyperhomocysteinemia [3, 19, 53]. An increase of H₂S content is observed in patients with Down's syndrome, with decompensated liver cirrhosis, sepsis, ischaemic stroke, chronic obstructive pulmonary diseases [19, 3].

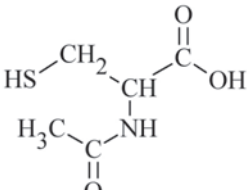
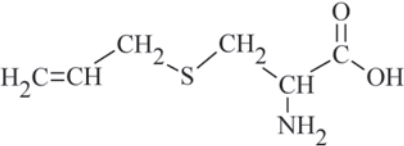
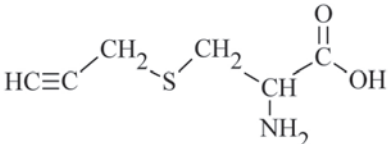
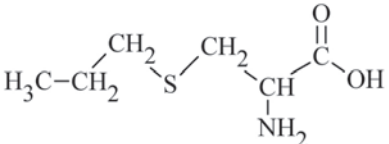
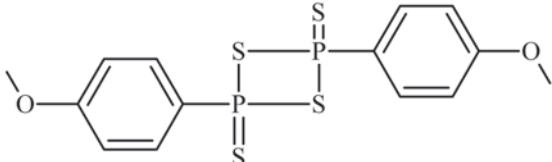
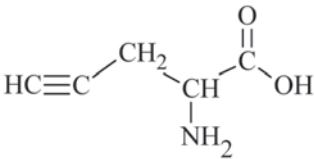
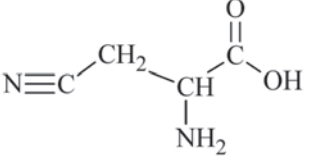

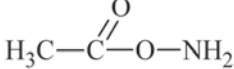
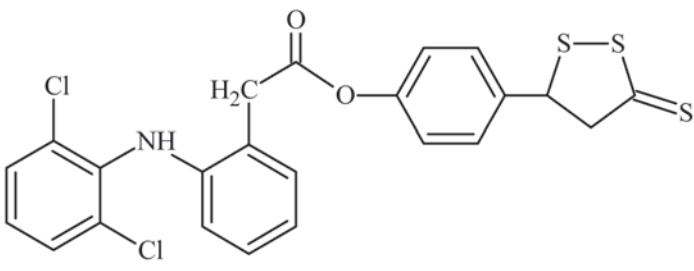
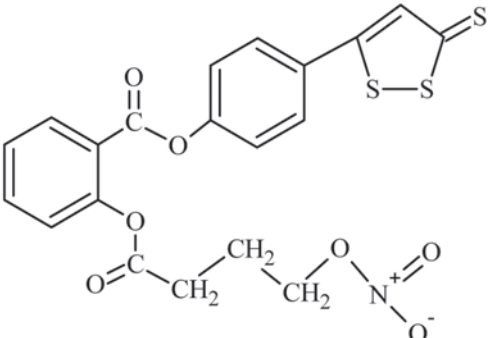
H₂S metabolism modulators in biology and medicine. The following basic approaches are used to study H₂S role *in vivo* and *in vitro*: 1) introduction of inorganic and organic H₂S donors; 2) introduction of specific inhibitors of H₂S-synthesizing enzymes; 3) modification of efficiency and toxicity of pharmacological remedies with the help of H₂S; 4) modification of H₂S metabolism with the help of pharmacological drugs. Examples of some H₂S metabolism modulators are presented in Table 7.

H₂S under conditions of organism generally works in the form of HS⁻, that is why NaHS or Na₂S are used as its donors. Information on doses, ways and duration of administration to animals of inorganic H₂S donors varies [80]. NaHS/Na₂S, according to most studies, are injected parenterally (intraperitoneally, intravenously) in doses from 100 μ g/kg to 3 mg/kg of the animal body weight, and duration of experiment may vary from several hours to 2–3 weeks [80]. In long-term studies (8–10 weeks) NaHS is added to drinking water in concentrations of about 30 μ mol/l; animals drink it *ad libitum* [81]. We should note that LD₅₀ for NaHS under parenteral injection is 14.6 \pm 1.0 mg/kg [82], in compliance with other data it is 60.2 mg/kg, and conventionally therapeutic doses of NaHS are about 1/20 LD₅₀ [83]. NaHS/Na₂S in such concentration range decrease the ischaemic-reperfusion damages under conditions of myocardial infarction, kidney ischemia, intestinal ischemia, show antioxidative, cerebroprotective, anti-inflammatory features [7, 17, 34, 68, 80, 81].

Vasodilatory, cytoprotective, antioxidative, anti-inflammatory effects of NaHS/Na₂S in studies *in vitro* are realized in broader range concentrations from 1 to 1000 μ M [80].

We have shown that H₂S donors demonstrate antiaggregation and anticoagulant effect: NaHS in concentration of 1 mM inhibited aggregation of human platelets, induced by ADP and collagen, and decreased amidolytic activity of thrombin (IC₅₀ = 65.3 \pm 3.76 μ M) *in vitro*; the injection of Na₂S·9H₂O (Sigma, USA) to rats in the amount of 3.36 mg/kg once a day during 7 days caused the increase of prothrombin time and activated partial thromboplastin time and decrease of Xa factor activity [19]. According to data of Nishikawa et al. (2013), NaHS inhibits thrombocyte aggregation, induced by ADP and collagen in plasma enriched by thrombocytes in concentrations of 0.1–0.3 mM, and in a suspension of washed up thrombocytes of rabbits in concentrations of 1–3 mM, respectively [84].

Table 7. Examples of H₂S metabolism modulators

H ₂ S donors and cysteine derivatives			
Na ₂ S	NaHS	 N-acetylcysteine	 S-allyl-L-cysteine
		 S-propargyl-L-cysteine	 S-propyl-L-cysteine
 Lawesson's reagent			
Inhibitors of H ₂ S-synthesizing enzymes			
 DL-propargylglycine	 β-cyanoalanine	 hydroxylamine	 aminooxyacetate
H ₂ S – derivatives NSAIDs			
 ACS 15 (S-diclofenac)		 NOSH-1 (aspirin-NO+H ₂ S)	

It has been showed in works of N. A. Strutynska, O. M. Semenykhina et al (2011, 2013) that NaHS or L-cysteine in physiological concentrations improve functional conditions of mitochondria in the heart,

prevent Ca²⁺-induced opening of mitochondrial pore and swelling of mitochondria at the time when CSE inhibitor propargylglycine increases sensitivity of mitochondria pore up to the action of Ca²⁺ inductor

and causes mitochondria swelling [102–104]. Gradual swelling of rats' heart's mitochondria is observed in potassium-free medium in the presence of NaHS in concentrations of 10^{-12} – 10^{-8} M [102]. Age distinctions regarding H_2S impact over pore creation in the heart mitochondria have been observed: NaHS protector effect in adult rats is realized in a more broad range of concentrations (10^{-6} , 10^{-5} and $5 \cdot 10^{-5}$ M) than in old rats (10^{-5} M) [102]. NaHS in the range of concentrations 10^{-9} – 10^{-6} M causes dose-depending decrease of oxygen absorption by isolated mitochondria of adult rats' heart in the presence of succinate and ADP at concentration of 10^9 and 10^{-8} M and increases connection between oxidation and phosphorylation [103]. Preincubation of isolated mitochondria with inhibitor of K^+ -ATP-channels 5-hydroxydecanoate significantly decreased the ability of NaHS (10^{-4} M) to prevent mitochondrial swelling under ischemia/reperfusion of myocardium [104].

Inhalation of H_2S aimed at cytoprotection is used in some studies, and concentrations of 50–400 ppm which cause mitochondrial dysfunction, are sublethal. H_2S in concentration up to 10 ppm does not create disorder in activity of mitochondrial enzymes [26]. The perfusion of lungs by gas mixture with H_2S in concentration of 50 and 100 $\mu\text{mol/l}$ decreased oxidative disorders at pulmonal ischemia/reperfusion in mice [80].

Inorganic H_2S donors quickly increase its content in blood plasma and tissues, but they are also quickly eliminated from the organism in a form of sulfides, thiosulfates, sulfites, sulfates. More slow increase of H_2S content in tissues provide H_2S -synthesizing enzymes substrates such as L-cysteine, D-cysteine; L-cysteine derivatives such as N-acetylcysteine, S-propyl-L-cysteine, S-propyl-L-cysteine, S-allyl-L-cysteine; polysulfides (diallyl disulfide, diallyl trisulfide) and artificial organic donor of H_2S - Lawesson's reagent [68, 85, 105]. Sodium thiosulfate may also play the role of inorganic substrate for endogenous H_2S production. For example, the injection of L-cysteine (15 and 100 mg/kg) stimulated epithelialization of gastric ulcer in rats [86]. D-cysteine protects neurons of cerebellum from oxidative stress and decreases damage of kidneys under condition of ischemia-reperfusion more effectively than L-cysteine [12]. S-propyl-L-cysteine, S-propyl-L-cysteine, S-allyl-L-cysteine showed cardioprotective action under acute myocardial infarction in rats, increased H_2S content and increased activity of superoxide dismutase (Mn-SOD) in cardiomyocytes

[105]. Administration of sodium thiosulfate (3 mg/ml with drinking water for 6 weeks) normalized cardiac H_2S production in rats with chronic heart failure [87].

To reach a decrease of H_2S endogenous production the H_2S -synthesizing enzymes have been used: inhibitors of CSE – DL-propargylglycine, β -cyanoalanine or inhibitors of CBS – hydroxylamine, aminooxyacetate. As a rule, inhibitors of H_2S -synthesizing enzymes demonstrate vasoconstriction, cytotoxic, pro-oxidant and pro-inflammatory action [7, 15, 17, 34, 68, 80, 81]. For example, the injection of DL-propargylglycine caused a decrease in activity of mitochondrial electron transport chain Complex I, decrease of glutathione content and development of oxidative stress in the brain of mice [36]; increased ischemia-reperfusion damages and increased size of myocardial infarction, worsened functional condition of kidneys and increased content of creatinine in blood serum of rats [80]. Seven-day injection of propargylglycine (50 mg/kg) caused activation of blood coagulation and increased ADP-induced aggregation of thrombocytes in rats [88]. With excessive H_2S in tissues, the inhibitors of H_2S -synthesizing enzymes may show cytoprotector action. Propargylglycine injection (8–10 mg/kg) decreased features of acute tubular necrosis, prevented the increase of serum creatinine and decreased H_2S creation in rats' kidneys under gentamicin administration [89].

Taking into consideration H_2S physiological effects, the attempts have been made to modify effectiveness and toxicity of medicines with the help of its donors. Thus there appeared H_2S -releasing drugs, and H_2S -derivatives of nonsteroidal anti-inflammatory drugs (NSAIDs) became their first example. It has been proved that one of the mechanisms of gastroduodenal toxicity of NSAIDs is a decrease of H_2S endogenous production [90]. H_2S -derivatives, S-diclofenac, S-naproxen, S-aspirin possess a higher anti-inflammatory action and lower gastrototoxicity than their prototypes [86, 91]. (H_2S +NO)-derivatives of aspirin and H_2S -derivatives of other pharmacological means – L-DOPA (ACS₈₃₋₈₆), sildenafil (ACS6) and mesalamin (ATB-429) were made later [68].

Pharmaceutical means may influence H_2S content in animals' organs in different ways. For example, paracetamol decreased H_2S concentration in brain, but increased its concentration in the heart, liver and kidneys of mice [92]. Amlopidin caused a decrease of H_2S level in the brain and liver of mice

in a dose of 3 and 10 mg/kg, but caused its increase in the heart and kidneys in a dose of 3 mg/kg and its decrease in a dose of 10 mg/kg [56]. It has been demonstrated as well that aspirin decreased H_2S level in the brain and increased its level in the liver of mice [93]. Carvedilol (in a dose of 10 mg/kg) caused an increase of H_2S content in the heart, kidneys and brain of mice [94]. The same effect was caused by digoxin [54], atorvastatin [92], ramipril [95], and metformin [96].

Medicines' influence on H_2S content in tissues, and their interaction with H_2S donors and inhibitors of H_2S -synthesising enzymes are not known well. It is proved that atorvastatin increases H_2S content in perivascular adipose tissue at the expense of inhibition of its mitochondria utilization, but does not influence the CSE activity. Atovastatin disrupts endogenous production of cofactor of sulfide quinone oxidoreductase – ubiquinone that significantly decreases its content in the blood plasma and tissues of animals [97]. Other drugs may be non-specific activators or inhibitors of H_2S -synthesizing enzymes. We have shown that a single injection of cisplatin (7 mg/kg) causes a significant decrease of CSE, CBS and CAT activity in the rat kidneys, that is associated with a decrease of H_2S level and increase of homocysteine level in the blood plasma. The injection of DL-propargylglycine (50 mg/kg) potentiated nephrotoxic effect of cisplatin, while NaHS injection (3 mg/kg), on the contrary, decreased cisplatin-induced nephropathy, increased glutathione content and glutamate-cysteine ligase in the rat kidneys [98].

It is not improbable that inorganic and organic donors of H_2S may significantly impact biotransformation of medicines. NaHS peroral administration (5 mg/kg) caused a decrease of CYP2C9 activity, increase of CYP3A4 activity and had no influence on CYP2B6, CYP2D6 and CYP2C19 activity in rats [99].

Search for safe and effective H_2S modulators is continued. In our opinion, the drugs containing activators and cofactors of H_2S -synthesizing enzymes, vitamin-microelements complexes, are perspective. We have showed in some studies that under long-term hyperhomocystenemia in rats H_2S content in the blood plasma and H_2S -synthesizing enzymes activity (CSE, CBS, CAT, TST) in the liver, kidneys, aorta is decreased. Administration of vitamin-microelement complex (VMC), which contains vitamins B_6 , B_9 , B_{12} and coordinating compositions of zinc (Zn^{2+}) and chrome (Cr^{3+}) with N-2,3-dimethyl-

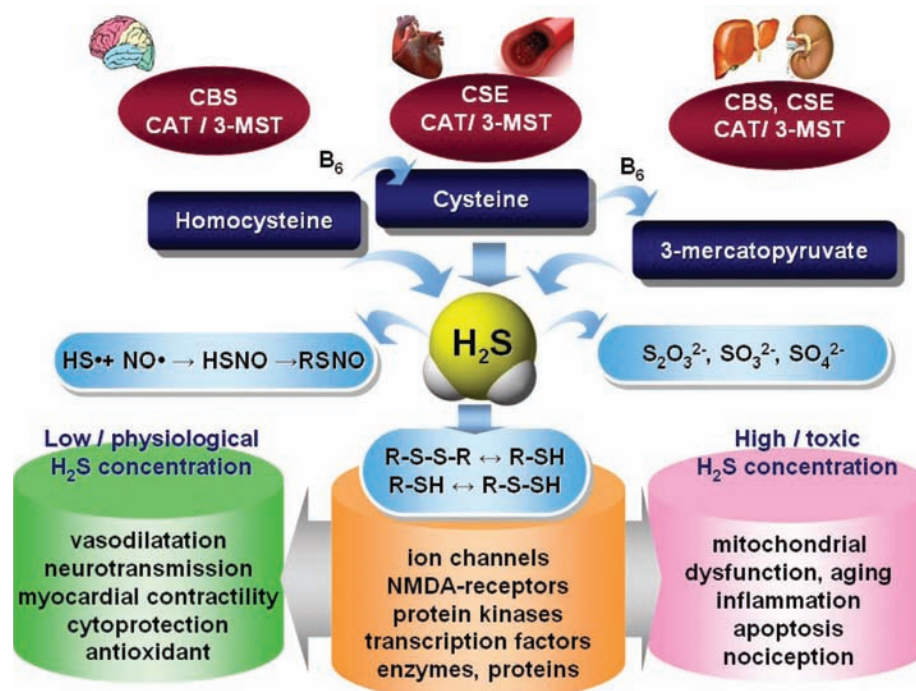
phenylanthranilic acid and ammonium vanadate (V^{5+}), effectively increased endogenous H_2S production from cysteine, homocysteine, thiosulfate in rats' organs. The H_2S production was also increased under the condition of combination of cysteine, homocysteine, thiosulfate in rats' organs with hyperhomocystenemia and also under its combination with nitric oxide synthase inhibitor L-NAME [57]. A complex of essential microelements (iron, copper, zinc, cobalt, manganese, chrome) and oxygen-containing salts of ultramicroelements (vanadium, molybdenum and selenium) – esmin (Esmin, PC "Kyiv Vitamin Factory") in a dose of 35 mg/kg decreased age-associated reduction of H_2S production in the myocardium, aorta and kidneys of rats [100]. The above-mentioned microelements are necessary for the broad range of biochemical processes; in particular, they are cofactors or activators of antioxidant enzymes and enzymes of sulfur-containing amino acids metabolism, involved in cardiovascular homeostasis and tissue respiration.

Thus a great body of scientific information about H_2S role in biology and medicine (Scheme) was accumulated within the last ten years. H_2S metabolism modulation has assumed great significance in determining mechanisms of formation of different pathological conditions, in development of new approaches to their preventive maintenance and correction, in increasing effectiveness and safety of pharmacotherapy and in development of many other directions, which amount increases. In our opinion, there is a necessity for systematization of H_2S metabolism modulators and creation of their working classification. We propose to divide modulators of H_2S metabolism, which are used in biology and medicine, into groups and subgroups in respect of their impact on endogenous H_2S in tissues and mechanism of their action (Table 8):

1) means that increase H_2S content in tissues (with regard to action mechanism, they are divided into H_2S donors; H_2S -synthesising substrates of enzymes and their derivatives; H_2S -releasing drugs; remedies containing cofactors and activators of H_2S -synthesizing enzymes; drugs inhibiting H_2S utilization);

2) means that decrease H_2S content in tissues (with regard to action mechanism, they are divided into specific and non-specific inhibitors of H_2S -synthesizing enzymes);

3) means with indeterminate mechanism of impact on H_2S metabolism. This group contains phar-



Scheme. Main directions of metabolism and biological effects of H₂S

Table 8. Classification of H₂S metabolism modulators, which are used in biology and medicine

Groups		Representatives
1. Agents that increase H ₂ S content in tissues		
1.1	H ₂ S donors	Inorganic – NaHS, Na ₂ S; organic – Lawesson's reagent
1.2	Substrates of H ₂ S-synthesizing enzymes and their derivatives	L-cysteine, D-cysteine, N-acetylcysteine, S-allyl-L-cysteine, S-propargyl-L-cysteine, S-propyl-L-cysteine, sodium thiosulfate
1.3	Agents with H ₂ S releasing effect (H ₂ S-releasing drugs)	H ₂ S-derivatives of non-steroidal anti-inflammatory drugs (S-aspirin, S-diclofenac, S-naproxen), L-DOPA (ACS83-86), sildenafil (ACS6)
1.4	Agents that contain cofactors and activators of H ₂ S-synthesizing enzymes	Vitamin-microelement and polymicroelement complexes (VMC and esmin)
1.5	Agents that inhibit H ₂ S utilization	atorvastatin
2. Agents that decrease H ₂ S in tissues		
2.1	Specific inhibitors of H ₂ S-synthesizing enzymes	DL-propargylglycine, β-cyanoalanine, β-aminoxyacetate, hydroxylamine
2.2	Nonspecific inhibitors of H ₂ S-synthesizing enzymes	non steroidal anti-inflammatory drugs (diclofenac, ketoprofen, indomethacin, aspirin), cisplatin
3. Agents with indefinite mechanism of action on H ₂ S metabolism		
3.1	Increase H ₂ S in tissues	carvedilol, digoxin, ramipril, paracetamol (in liver, kidneys), metformin, amlodipin
3.2	Decrease H ₂ S in tissues	paracetamol (in brain), amlodipin

macology means, influence of which on H_2S metabolism necessitates further study.

It is obvious that the proposed classification of H_2S will change with the extension of the range of its representatives, with determination and clarification of their action mechanisms. There are many unsolved problems concerning molecular targets, ways of H_2S -signaling realizations, regulation mechanisms of H_2S synthesis and degradation under the effect of different endogenous and exogenous factors. Solution of these problems opens new prospects in development of medical biochemistry and pharmacology.

ГІДРОГЕНСУЛЬФІД: МЕТАБОЛІЗМ, БІОЛОГІЧНЕ ТА МЕДИЧНЕ ЗНАЧЕННЯ

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Гідрогенсульфід (H_2S) є сигнальною молекулою, яка активно синтезується в тканинах і бере участь у регуляції судинного тону, нейромодуляції, цитопротекції, в запаленні, апоптозі. В останні роки накопичилися нові дані про метаболізм та функції H_2S в організмі тварин та людини в умовах дії різних ендогенних та екзогенних чинників, у тому числі і лікарських засобів. У представленому огляді узагальнено інформацію про основні та альтернативні шляхи метаболізму H_2S та їх регуляцію, особливості його транспортування, сигналіngu, біологічну роль, участь в розвитку патологічних станів. Наведено дані щодо вмісту H_2S та активності H_2S -синтезуючих ензимів у різних органах, щодо впливу H_2S на процеси зсідання крові та агрегації тромбоцитів з урахуванням результатів власних досліджень. Запропоновано робочу класифікацію модюляторів обміну H_2S , які використовуються в біології та медицині: 1) засоби, що підвищують вміст H_2S у тканинах (неорганічні та органічні донори H_2S ; субстрати H_2S -синтезуючих ензимів та їх деривати; засоби з ефектом вивільнення H_2S ; засоби, що містять кофактори та активатори H_2S -синтезуючих ензимів; засоби, які інгібують

утилізацію H_2S); 2) засоби, що знижують вміст H_2S у тканинах (специфічні та неспецифічні інгібітори H_2S -синтезуючих ензимів); 3) засоби з невизначеним механізмом впливу на обмін H_2S (окремі фармакологічні засоби). Показано, що перспективними засобами для корекції вмісту H_2S у тканинах є вітамінно-мікроелементні та мікроелементні комплекси, які містять кофактори та активатори H_2S -синтезуючих ензимів.

Ключові слова: гідрогенсульфід, H_2S -сигналіng, ензими, регуляція, модюлятори обміну H_2S , комплекси вітамінів та мікроелементів.

ГИДРОГЕНСУЛЬФИД: МЕТАБОЛИЗМ, БИОЛОГИЧЕСКОЕ И МЕДИЦИНСКОЕ ЗНАЧЕНИЕ

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Гидрогенсульфид (H_2S) является сигнальной молекулой, которая активно синтезируется в тканях и участвует в регуляции сосудистого тонуса, нейромодуляции, цитопротекции, воспалении, апоптозе. В последние годы накопились новые данные о метаболизме и функции H_2S в организме животных и человека под влиянием различных эндогенных и экзогенных факторов, в том числе и лекарственных средств. В представленном обзоре обобщена информация об основных и альтернативных путях метаболизма H_2S и их регуляции, особенности его транспорта, сигналинга, биологической роли, участие в развитии патологических состояний. Приведены данные о содержании H_2S и активности H_2S -синтезирующих энзимов в различных органах, о влиянии H_2S на процессы свертывания крови и агрегации тромбоцитов с учетом результатов собственных исследований. Предложена рабочая классификация модюляторов обмена H_2S , которые используются в биологии и медицине: 1) средства, повышающие содержание H_2S в тканях (неорганические и органические доноры H_2S ; субстраты H_2S -синтезирующих энзимов и их дериваты, средства с эффектом высвобождения H_2S ; средства, содержащие кофакторы и активаторы H_2S -синтезирующих энзимов; сред-

ства, ингибирующие утилизацию H_2S), 2) средства, снижающие содержание H_2S в тканях (специфические и неспецифические ингибиторы H_2S -синтезирующих энзимов), 3) средства с неопределенным механизмом влияния на обмен H_2S (отдельные фармакологические средства). Показано, что перспективными средствами для коррекции содержания H_2S в тканях является витаминно-микроэлементные и микроэлементные комплексы, содержащие кофакторы и активаторы H_2S -синтезирующих энзимов.

Ключевые слова: гидрогенсульфид, H_2S -сигналинг, энзимы, регуляция, модуляторы обмена H_2S , комплексы витаминов и микроэлементов.

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