THE FATE OF SULFIDES IN THE BLOOD.*

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The investigation embodied in this paper was primarily undertaken in an endeavor to determine in what manner H₂S is transported in the blood after inhalation of this gas. That it is transported in the blood in a loosely combined and readily dissociable manner is evident from the fact that, when H₂S is injected into any of the body cavities, it is almost at once detectable in the expired air (1). On the question in what form hydrogen sulfide combines with and is carried by the blood there is diversity of opinion, however.

Two modes of transportation have been suggested: That the hydrogen sulfide combines with the hemoglobin to form a sulf-hemoglobin compound; and that the gas in its capacity as an acid unites with the alkali of the blood to form sodium sulfide. Since both of these combinations are dissociable both views necessarily assume a trace of H₂S in simple solution in the plasma.

Paradoxically, although the first of these views is definitely disproved by evidence contained in the literature, the belief that inhalation of H₂S is followed by the formation of a sulfur hemoglobin compound is still generally prevalent. The persistence of this idea is no doubt due in part at least to the need for an explanation of the rare disease, sulfhemoglobinemia, one of the endogenous cyanoses.

* This is one of a series of papers dealing with hydrogen sulfide poisoning. Other papers of this series will probably appear in the American Journal of Physiology, the Journal of Pharmacology and Experimental Therapeutics, and the Journal of Industrial Hygiene.
The reaction of hemoglobin to H₂S has been the subject of much study. Hoppe-Seyler (2) in 1863 and later Araki (3), by passing a stream of H₂S through oxygenated blood, obtained a dirty greenish pigment. It showed an absorption spectrum with two bands in the red, one quite close to C and the other midway between C and D. This compound was designated sulfmethemoglobin. The green discoloration seen in cadaveric decomposition was assigned to the postmortem formation of sulfmethemoglobin.

Gamgee (4) in 1898 branded the sulfmethemoglobin of Hoppe-Seyler and Araki as a mixture of decomposition products. Harnack (5), a year later, while also emphatically denying the existence of this substance, obtained in solution by the action of H₂S upon reduced hemoglobin, a compound which he designated as sulfhemoglobin. Its absorption spectrum gave but a single band between the Fraunhofer lines C and D and extending from λ.610 to λ.625.

In 1907, Clarke and West (6) verified Harnack's work and attempted to isolate the compound, but were unsuccessful. They noted that complete reduction of the hemoglobin was essential to the formation of this substance. They found further, that very high concentrations of H₂S were necessary to force a union between hemoglobin and this gas. These concentrations were far higher than those which would be instantly fatal if inspired. The presence of powerful reducing agents, such as phenylhydrazine, however, greatly facilitated the reaction and rendered a partial combination possible at relatively low concentrations of H₂S and even in the presence of oxyhemoglobin.

It is on the basis of this last observation that the occurrence, during life, of blood giving the spectrum of sulfhemoglobin—the characteristic of the disease known as sulfhemoglobinemia—is explicable. Wallis (7) found that the formation of sulfhemoglobin in the blood of patients suffering from this disease, is due to the presence in their blood of a strong reducing agent, a hydroxylamine derivative, which presumably comes from the splitting of protein by a nitrosobacillus which inhabits the buccal cavities. A mere trace of H₂S is necessary under these conditions to form sulfhemoglobin and this might be derived from the intestinal tract.
From these facts it is apparent that the formation of sulfhemoglobin within the living body is primarily dependent upon an abnormal condition of the blood, rather than upon any function of the hemoglobin as the transporting agent of H₂S. Within the body of a healthy living individual—one whose mouth does not contain this nitrosobacillus—complete reduction of the blood would be essential before any combination of the gas with hemoglobin could occur.

As a postmortem change, however, sulfhemoglobin may be rapidly formed through bacterial action. This change, we may presume, consists first in a reduction of the blood and then a combination of the hemoglobin with the H₂S liberated during the process of decomposition.

It is a highly significant fact, that the blood taken very soon after death from H₂S poisoning does not show the spectrum characteristic of sulfhemoglobin and is abnormal, if at all, only in the degree of its reduction (7, 8). *A fortiori* hemoglobin is not the normal transporter of H₂S.

Diakonow (9) was apparently the first to point out that, through its properties as an acid, H₂S should act upon the bicarbonates of the blood plasma to form sodium sulfide. He demonstrated this experimentally upon bicarbonate solutions. Following this lead Pohl (10) came to the conclusion that H₂S must be transported in the blood in this form alone. In support of this view he pointed out the remarkable similarity between poisoning with H₂S gas and that induced by injection of sodium sulfide.

It seems plausible that, to some extent at least, sodium sulfide would be formed within the plasma after inhalation of this gas. For this reason the following experiments were undertaken. In the main, however, they have led to quite another conclusion.

**EXPERIMENTAL.**

The following experiments which were repeatedly performed, show a marked difference between the reactions toward H₂S of sodium bicarbonate solution on the one hand and of plasma on the other. The former combines with the gas to some extent, presumably as Na₂S and even when washed free of dissolved H₂S gives a persistent test for sulfide. The plasma, however, retains
no sulfide, detectable as such, in any form other than the dissolved 
H₂S gas. Evidently plasma after exposure to a moderate amount 
of H₂S does not contain Na₂S.

Experiment 1. The Action of H₂S upon Sodium Bicarbonate Solution.—
5 cc. of 0.2 per cent sodium bicarbonate solution were shaken in a flask con-
taining an atmosphere of 0.5 per cent H₂S in air. The flask was then 
opened and air passed through until the odor of H₂S was no longer detected. 
The solution was then tested, and gave a sulfide reaction both with lead 
acetate and with ammoniacal sodium nitroprusside. The passage of pure 
air, 5.5 per cent CO₂ in air, or oxygen through the liquid for 3 hours did 
not render it incapable of giving a sulfide test. The addition of dilute 
HCl to the fluid was attended with the evolution of H₂S.

Experiment 2. The Action of H₂S upon Plasma.—Dog’s plasma was 
shaken in a flask with 0.5 per cent H₂S and then aerated. The plasma 
failed to give any of the above tests for sulfide nor was H₂S evolved upon 
the addition of dilute HCl.

This experiment with plasma (Experiment 2) affords a clear-cut 
and decisive negative on the question to which it is primarily 
directed. The striking difference from the result with bicar-
bonate solution (Experiment 1) prompts the further question as 
to just what does happen when H₂S is brought into contact with 
plasma.

To investigate this question, further experiments were there-
fore performed. Plasma was exposed to a definite volume of 
H₂S in air, nitrogen, CO₂, or oxygen and then washed free of 
dissolved H₂S with the same atmosphere. The hydrogen sulfide 
recoverable from the gas was estimated quantitatively. In some 
cases the CO₂-combining power of the plasma before and after 
treatment with H₂S was determined as a means of following any 
change in the sodium bicarbonate of the plasma.

Experiment 3. The Reaction between H₂S and Plasma or Blood.—The 
CO₂-combining power of a sample of normal plasma from dog’s blood 
was determined at 40 mm. partial pressure CO₂. Some of the plasma was 
then evacuated of gas by means of a suction pump, and 5 cc. samples were 
pipetted into 1 liter flasks containing, in successive tests, atmospheres of 
air, oxygen, nitrogen, and 40 mm. CO₂ in air. To all of these atmospheres, 
0.5 per cent H₂S had been added. Each flask was rotated for 1 minute. 
The H₂S which could be recovered was then determined by aerating the 
flask with the same atmosphere with which it was filled, minus the H₂S, 
and drawing the gas through a bead tower containing 0.01 N iodine (11).
In each case at the end of the aeration the plasma was tested for sulfide; but only negative results were obtained.

Samples of whole blood were treated in the same manner except that the initial evacuation was omitted. Table I embodies the results obtained.

From Experiment 3 it appears that not only does H$_2$S fail to form sodium sulfide when acting upon blood or plasma but that a portion of the gas is actually destroyed. The destruction of

<table>
<thead>
<tr>
<th>Atmosphere in flask</th>
<th>CO$_2$-combining power of plasma at 40 mm. partial pressure</th>
<th>H$_2$S recovered</th>
<th>H$_2$S lost</th>
<th>H$_2$S oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before exposure</td>
<td>After exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction between plasma and H$_2$S.</td>
<td>vol. per cent</td>
<td>vol. per cent</td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>Air and 5.2 cc. of H$_2$S.</td>
<td>54</td>
<td>40</td>
<td>3.10</td>
<td>2.10</td>
</tr>
<tr>
<td>&quot; &quot; 4.6 &quot; &quot; H$_2$S.</td>
<td>54</td>
<td>46</td>
<td>2.94</td>
<td>1.66</td>
</tr>
<tr>
<td>&quot; &quot; 5.4 &quot; &quot; H$_2$S.</td>
<td>54</td>
<td>41</td>
<td>3.32</td>
<td>2.08</td>
</tr>
<tr>
<td>&quot; with CO$_2$ at 40 mm. and 5.7 cc. of H$_2$S.</td>
<td>54</td>
<td>45</td>
<td>3.42</td>
<td>2.28</td>
</tr>
<tr>
<td>&quot; &quot; CO$_2$ &quot; 40 &quot; &quot; 5.5 &quot; &quot; H$_2$S.</td>
<td>54</td>
<td>44</td>
<td>3.70</td>
<td>1.8</td>
</tr>
<tr>
<td>Oxygen and 5.6 cc. of H$_2$S.</td>
<td>2.11</td>
<td>4.49</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; 5.4 &quot; &quot; H$_2$S.</td>
<td>2.4</td>
<td>4.0</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Nitrogen and 5.0 cc. of H$_2$S.</td>
<td>4.60</td>
<td>0.40</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; 5.1 &quot; &quot; H$_2$S.</td>
<td>4.42</td>
<td>0.68</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

H$_2$S is here seen to be dependent upon the presence of oxygen and is, therefore, an oxidation. The oxidized products combine in part with the alkali of the plasma and decrease its CO$_2$-combining power.

Obviously this raises the question whether the loss in alkali, as measured by the CO$_2$-combining power of the plasma, is adequate to account for the amount of H$_2$S destroyed on the basis of a bivalent combination of sulfur (H$_2$SO$_4$) with the sodium. Cal-
culation shows, however, that the oxidation of 1 cc. of H₂S would produce enough sulfuric or other bivalent acids to reduce the CO₂ capacity of 5 cc. of blood or plasma by 20 volumes per cent. The figures actually obtained are only 20 to 30 per cent of this amount. A portion of the oxidation products must, therefore, be in combination with constituents of the blood other than alkali, or otherwise concealed.

In oxygenated whole blood the oxidation of hydrogen sulfide is more complete than in plasma. This is due apparently not to a catalytic action of the corpuscles, but to the greater available supply of oxygen. The withdrawal of oxygen from the corpuscles in the oxidation of hydrogen sulfide results in the deoxygenation of the blood. This process affords the explanation of the reduction of hemoglobin by H₂S, which has been observed by previous workers (6).

Not only does the plasma fail to combine H₂S with alkali to form sodium sulfide but, as will be seen in Experiment 4, plasma has the property of dissociating sodium sulfide and liberating H₂S from it. The absence of oxygen has no inhibitory influence upon this reaction. In the presence of oxygen, however, a portion of the liberated H₂S is oxidized. The processes of dissociation and oxidation are to some extent distinct and independent each from the other. The first is apparently of the nature of a catalytic acceleration of the well recognized and easily demonstrated reaction of sodium sulfide with water:

\[ \text{Na}_2\text{S} + 2\text{H}_2\text{O} = \text{H}_2\text{S} + 2\text{NaOH}. \]

The oxidation on the other hand possesses many of the characteristics of oxidative reactions which occur through the action of tissue juices. The importance of the part played by the SH group in vital oxidations is beginning to attract particular attention (12).

The whole phenomenon of hydrolysis and oxidation can be illustrated quite simply by an experiment designed by Clarke and West (6) to demonstrate the reduction of blood by sodium sulfide. To the bottom of a test-tube of blood is passed a small amount of a strong solution of sodium sulfide. Gradually the area above the sulfide becomes reduced and this reduction progresses up the tube. It is a highly significant fact that even before the
upper layer changes color the odor of the liberated and unoxidized \( \text{H}_2\text{S} \) can be detected.

In this or any similar experiment the sodium sulfide added to the blood or plasma becomes completely hydrolyzed, and with adequate aeration the \( \text{H}_2\text{S} \) thus formed is either oxidized or volatilized off. Thereafter the blood or plasma does not respond to tests for sulfides. As would be anticipated, one result of the hydrolysis of the sulfide is that the sodium increases the alkali in the fluid and raises its \( \text{CO}_2 \)-combining power. If the oxidation is active a portion of the liberated sodium is used up in combining with the products of oxidation, presumably in part at least sulfuric acid.

Experiment 4. The Reaction of Sodium Sulfide with Blood and Plasma.—Samples, 5 cc. each, of dog's plasma were evacuated of gas, and then placed in flasks containing atmospheres of air, oxygen, or nitrogen. 3 cc. of approximately 0.8 per cent sodium sulfide solution were added. The aerating gas was then passed through the mixture, and the \( \text{H}_2\text{S} \) carried over was absorbed in 0.01 \( \text{N} \) iodine and calculated as in the previous experiment. The amount of \( \text{H}_2\text{S} \) recoverable when acidulated water was used instead of blood was 6.1 cc.; this figure, 6.1, has been used throughout as a control. After the plasma had been washed free of \( \text{H}_2\text{S} \), the fluid was tested for sulfide, with negative results in all cases. The whole blood used was treated in a manner similar to the plasma except that the initial exhaustion was omitted. Table II embodies the results obtained.

<table>
<thead>
<tr>
<th>Atmosphere in flask</th>
<th>( \text{H}_2\text{S} ) recovered.</th>
<th>( \text{H}_2\text{S} ) lost.</th>
<th>( \text{H}_2\text{S} ) oxidized.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air...</td>
<td>2.9 cc.</td>
<td>3.2 cc.</td>
<td>53 per cent</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.6 cc.</td>
<td>3.5 cc.</td>
<td>57 per cent</td>
</tr>
<tr>
<td>&quot;</td>
<td>3.0 cc.</td>
<td>3.1 cc.</td>
<td>51 per cent</td>
</tr>
<tr>
<td>Nitrogen...</td>
<td>5.9 cc.</td>
<td>0.2 cc.</td>
<td>3 per cent</td>
</tr>
<tr>
<td>&quot;</td>
<td>5.6 cc.</td>
<td>0.5 cc.</td>
<td>8 per cent</td>
</tr>
</tbody>
</table>

Table II. The reaction of sodium sulfide with plasma.

<table>
<thead>
<tr>
<th>Atmosphere in flask</th>
<th>( \text{H}_2\text{S} ) recovered.</th>
<th>( \text{H}_2\text{S} ) lost.</th>
<th>( \text{H}_2\text{S} ) oxidized.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air...</td>
<td>1.2 cc.</td>
<td>4.9 cc.</td>
<td>80 per cent</td>
</tr>
<tr>
<td>&quot;</td>
<td>1.6 cc.</td>
<td>4.5 cc.</td>
<td>74 per cent</td>
</tr>
<tr>
<td>Oxygen...</td>
<td>1.0 cc.</td>
<td>5.1 cc.</td>
<td>83 per cent</td>
</tr>
</tbody>
</table>

Experiment 4 illustrates the hydrolysis of sodium sulfide by plasma or whole blood and the oxidation of the liberated \( \text{H}_2\text{S} \) in the presence of oxygen.
The hydrolyzation of Na$_2$S by plasma and the subsequent oxidation of a portion of the liberated H$_2$S is extremely rapid. This is seen in the following experiment which was designed primarily to determine whether or not the products of oxidation were toxic.

**Experiment 6. The Detoxication of Sodium Sulfide by Plasma.**—To 10 cc. of dog's plasma was added 0.6 gm. of Na$_2$S in 10 per cent water solution and the whole shaken for 1 minute with a stream of air from the blower passing through the flask. At the end of this time 1 cc. of the mixture was tested with negative results for the presence of sulfide. The rest of the plasma thus treated was injected intravenously into a 10 kilo dog. The inoculation was entirely without effect upon the animal although the amount of Na$_2$S added to the plasma was ten times the ordinary lethal dose.

From this experiment we may conclude that the oxidation products of H$_2$S or sodium sulfide in the blood are non-toxic. Evidently this process of detoxication may be of considerable importance during the putrefaction of sulfur-containing substances in the intestine. Heretofore it has usually been ascribed to the liver.

In consideration of this point it would seem that when H$_2$S is inhaled it exists only momentarily in the blood as the dissolved and unoxidized gas. Of course, theoretically, an infinitesimal trace of alkali sulfide must be present. The active physiological effects of H$_2$S are exerted by the gas in solution in the plasma.

In view of the oxidative process above illustrated in the *in vitro* experiments, it is obvious that *in vivo* the gas can exhibit no cumulative action when inhaled. This is well borne out by other experimental findings, for in observations to be reported in detail in a later paper, it has been found that the inhalation of 5 parts of H$_2$S in 10,000 parts of air for a period of 11 hours produces no general symptoms in dogs, while the inhalation of 10 parts results in death in 15 minutes.

If the effects were cumulative there would not be this wide variation in the effects of such closely related concentrations. The rapid recovery from non-fatal inhalational poisoning by H$_2$S is a striking confirmation of the lack of cumulative action of this gas and of the necessity for the maintenance in the blood of a toxic concentration in order to produce the characteristic symptoms of poisoning. An animal removed from an atmosphere
of $\text{H}_2\text{S}$ in a state of deep coma, frequently appears quite normal after the lapse of a few minutes. The same considerations apply to the effects following intravenous injection of a solution of sodium sulfide. The salt is hydrolyzed in the blood and $\text{H}_2\text{S}$ is at once apparent in the expired air. If the administration is rapid enough to allow a sufficiently high concentration of unoxidized $\text{H}_2\text{S}$ in the blood, toxic symptoms and death result.

These points are illustrated in Experiment 6 in which, by slow intravenous injection, many times the lethal amount of sodium sulfide was administered. According to the literature the lethal dose of sodium sulfide for dogs is 6 mg. per kilo, and this has been confirmed here.

**Experiment 6. The Repeated Administration of Sodium Sulfide.**—A 10 kilo dog was subjected to intravenous injection of sodium sulfide solution (0.6 per cent) at the rate of 2.5 cc. per minute. (This is 25 per cent of the lethal dose each minute.) The animal exhibited some restlessness and a slight dyspnea but, after a total injection of 50 cc. during 20 minutes, was apparently none the worse, although five times the lethal amount had been administered.

A rapid injection of 10 cc. of the solution was then made. After a few gasps the animal became rigid and died.

This experiment also throws some light upon the comparatively slight toxic effects induced by the sulfide formed in the intestine from the decomposition of protein or following the administration of sulfur. The sulfide so formed is slowly absorbed, and completely detoxicated in the blood. Large amounts of sulfur may be thus altered and eliminated without the development of any marked physiological effects although, following the ingestion of sulfur, the breath may be foul with $\text{H}_2\text{S}$. In this connection it may be mentioned that about 10 per cent of the sulfur taken by mouth is absorbed as sulfide and later eliminated through the urine as sulfates and in organic combination (13). There are, however, a few reported cases of toxic symptoms following the still prevalent administration of sulfur (14).

Since the capacity of the blood to oxidize $\text{H}_2\text{S}$ is quite adequate to cope with many times the lethal amount, it is necessary to offer some explanation of the intense physiological activity of this gas which when inhaled ranks close to cyanogen in toxicity (14). The laws of mass action offer a tenable explanation of the phe-
nomena. The dissolved \( \text{H}_2\text{S} \) in the blood is a factor in the equilibrium of the reaction of oxidation. The greater the amount of inhaled \( \text{H}_2\text{S} \) the more active will be the oxidation, but there will be also momentarily a higher concentration of \( \text{H}_2\text{S} \) dissolved in the blood and in consequence a greater physiological effect.

Unless death supervenes the concentration of \( \text{H}_2\text{S} \) dissolved in the blood can only be maintained by the continued inhalation of the gas. When the inhalation ceases the \( \text{H}_2\text{S} \) present in the blood is rapidly and completely destroyed. This chemical process corresponds with the prompt recovery of the man or animal on removal from the atmosphere of \( \text{H}_2\text{S} \).

The assumption that the dissolved \( \text{H}_2\text{S} \) in the blood exists as an equilibrium factor in the oxidative reaction is supported by an experimental observation. When 10 or 15 cc. of \( \text{H}_2\text{S} \) gas (too small an amount to produce any symptoms, whatever) are injected into the peritoneal cavity of a dog, there soon follows the characteristic smell of the gas in the breath of the animal. When the active oxidation of \( \text{H}_2\text{S} \) shown by the blood in Experiment 3 is considered, it is indeed remarkable that a portion of the gas should escape unoxidized from the lungs, after passing through the venous system where there is certainly sufficient oxygen to destroy it, unless this was due to the persistence of the rapidly decreasing factor, concentration of \( \text{H}_2\text{S} \), in the trend back to equilibrium.

CONCLUSIONS.

When an atmosphere containing \( \text{H}_2\text{S} \) is inhaled no combination of the gas is formed with the hemoglobin of the blood nor is any appreciable amount of sodium sulfide formed in the plasma. The phenomena of the disease of sulfhemoglobinemia have no significance for the normal transport of sulfide.

Blood plasma in the presence of oxygen possesses the property of rapidly oxidizing \( \text{H}_2\text{S} \). The products of oxidation combine in part with the sodium of the plasma.

Sodium sulfide is rapidly and completely hydrolyzed by blood or plasma. The absence of oxygen has no effect upon this process. If oxygen is present, however, a large part of the liberated \( \text{H}_2\text{S} \) is oxidized.
The reduction of blood by H₂S or Na₂S is the result of the withdrawal of oxygen from the corpuscles for the oxidation of the H₂S.

After inhalation of H₂S or intravenous administration of Na₂S, the sulfide in the blood exists only as dissolved and as yet unoxidized H₂S. The active physiological effects of sulfides are exerted by the H₂S in solution in the blood. During the administration of sulfide in any manner the H₂S in solution in the blood is a factor in the reaction of oxidation.

The rate of oxidation of H₂S in the blood is such that in a comparatively short period many times the lethal amount of sodium sulfide may be administered intravenously to animals without any apparent effect. This explains the comparatively slight toxic properties exhibited by the absorption of sulfides from the intestinal tract.

In conclusion I wish to express my sincere thanks to Professor Yandell Henderson for suggestions and criticism.

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