LXII. THE PRODUCTION OF HYDROGEN SULPHIDE BY ANIMAL TISSUES.

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(Received February 21st, 1930.)

DE REY PAILHADE [1888] demonstrated that yeast and various animal tissues could reduce sulphur to hydrogen sulphide, and ascribed this activity to the presence of a specific constituent which he called philothion. His views as to its precise nature have several times undergone change, but at one time at least [1894] he ranked it as an oxidation ferment. The view that an enzyme is concerned in the phenomena has been contested, among others, by Abelous and Ribaut [1903], and especially by Heffter, who [Heffter and Hausmann, 1904, 1908] decided that the reducing action of organs on sulphur depends upon the influence of SH groups. Heffter, as is well known, developed views which attached great importance to the thiol group of cysteine in the respiration of tissues.

Strassner [1910] showed that the presence of SH plays a part in the reduction of methylene blue by the tissues; but the extensive experiments of Thunberg and his colleagues, as well as those of other workers, have demonstrated how large a part is played by the dehydrogenases in the reduction of substances such as methylene blue. On the other hand, Hopkins [1921, 1922, 1925] finds that dehydrogenases in the presence of their substrates do not apparently reduce cystine or oxidised glutathione. The power of the tissues to reduce these depends upon other factors. It would seem of interest to decide whether nevertheless these enzyme systems can reduce free S itself. While it is certain that the presence of SH groups is largely responsible for the production of H_2S from S it is not yet certain that enzyme activity, calling upon other sources of hydrogen, takes no share in the process.

To decide this point was the aim of the present investigation. Experiments have been carried out to determine (1) whether the same proportionate relation exists between the amount of SH in the tissues and the amount of the H_2S produced therefrom as obtains when solutions of pure glutathione are similarly treated; (2) the influence of temperature and p_H on the process; (3) the influence of H-donator concentration; and (4) the effect of adding a dehydrogenase.

METHODS USED.

It is well known that a solution of H_2S , made acid with HCl , gives, on the addition of dimethyl-*p*-phenylenediamine sulphate and a small amount of ferric chloride, methylene blue. Upon this reaction a colorimetric method for the estimation of H_2S may be based. In applying this method in my own experiments I have employed not a colorimeter, but an extincitometer as described by Moll. The procedure will be clear from the diagram (Fig. 1). A source of light (a 4-volt lamp) is placed between two thermopiles. Between the lamp and pile I is placed a trough containing the solution of which the heat adsorptive capacity is to be tested; between pile II and the lamp is placed an exactly similar trough containing water. The amount of heat which reaches the respective thermopiles and the corresponding potential produced varies of course with the adsorption exerted by the interposed solutions. The adsorption of the methylene blue solution under study is thus balanced against pure water as a control. The thermopiles are connected with a galvanometer as shown. *AB* and *CD* are symmetrically disposed resistances and the latter being adjustable is employed as a compensator.

Fig. 1. Extincitometer.

The apparatus is first adjusted by placing the lamp in such a position that both piles receive the same amount of radiation when both troughs are filled with water and the resistances equal. The galvanometer then registers no current. When in trough I the water is replaced by the coloured solution there is greater heat adsorption and the consequent diminution of the current from pile I is registered by the galvanometer. Equilibrium is then restored by shifting the contacts of *CD*. Decrease of the distance between *C* and *D* is proportional to a decrease in the thermoelectric current from pile II. The reading in ohms on the scale of *CD* gives, when the apparatus is calibrated, a direct measure of the concentration of methylene blue in trough I.

In order to test the method, varying known concentrations of methylene blue were placed in trough I and the readings on the compensator scale were plotted against these. Duplicate determinations agreed well (Table I) and the results yielded a smooth curve (Fig. 2).

Table I.

cc. of methylene blue sol. in 20 cc. water	Ohms
0.25	4.6; 4.6
0.50	7.2; 7.1
1.00	10.0;
2.00	12.8; 12.8

Fig. 2. Extinction curve of methylene blue.

There is, however, no relation between the extinctions of solutions of pure methylene blue and the solutions containing HCl as prepared from H₂S on the lines described. The spectrum of methylene blue has two bands in the red while that of its hydrochloride has but one; that on the boundary between red and yellow being absent from the latter. The colour of a solution containing HCl has therefore an element of orange. Since yellow absorbs more heat than violet less heat is transmitted by a given concentration of methylene blue when HCl is present.

The results given in Table II show this effect, the figures being given in the form of the compensator readings. It will be seen that the reagents added for the production of methylene blue from the H₂S have only a negligible heat absorption.

Table II.

Methylene blue 1/5000 cc.	Water cc.	HCl. cc.	Reagents cc.	Ohms
5	10.5	0	0	19.6
5	8	2	0.5	29.9
5	8.5	2	0	29.0
5	10	0	0.5	20.3
0	13	2	0.5	0.7

In order to calibrate the apparatus for use it was therefore necessary to construct a curve from methylene blue solutions as obtained by adding the reagents to H₂S solutions of known concentration, the procedure being the same as in the actual experiments. The H₂S solutions were made by diluting a solution very accurately titrated with iodine. The curve thus obtained is given in Fig. 3.

As a further test of the consistency of the results determinations by the method were made on each of two solutions which were then mixed, and determinations again made on the mixture. It will be seen (Table III) that the results are consistent.

Fig. 3. Calibration curve for H_2S .

Table III.

Solution	H_2S estimated mg.	Average mg.	H_2S deter- mined in mixture mg.
<i>a</i>	0.62	0.405	0.42
<i>a'</i>	0.19		
<i>b</i>	0.91	0.72	0.74
<i>b'</i>	0.53		
<i>c</i>	0.81	1.04	1.05
<i>c'</i>	1.28		
<i>d</i>	0.28	0.20	0.20
<i>d'</i>	0.12		
<i>e</i>	0.09	0.07	0.065
<i>e'</i>	0.05		

A number of experiments were made to determine the effect of temperature changes upon the result. It was found that within the ordinary range of room temperatures the effect was small though not negligible. It is best to work near to the temperature at which the apparatus is calibrated. Keeping the methylene blue solutions for 24 hours before making the estimation was found to have no effect upon the results.

THE YIELD OF H_2S WHEN SULPHUR IS BROUGHT IN CONTACT WITH GLUTATHIONE.

Hopkins has mentioned incidentally that reduced glutathione when shaken with sulphur yields H_2S . I have to thank Sir F. G. Hopkins for a supply of that substance sufficient for the following experiments.

I first set out to determine whether the thiolpeptide yields amounts of H₂S proportionate to the concentration of SH in solution. Solutions of various concentrations were made, the SH being determined by titration with *N*/100 iodine. The *p*_H of all solutions was adjusted to 7·6. To each was added a measured quantity of a 2 % solution of colloidal sulphur. The solutions were placed in bottles which were evacuated and placed in a thermostat for 2 hours. At the end of a given period the H₂S was in each case drawn over by a stream of air which had been washed through lead acetate solution into a known volume of *N*/10 NaOH. To the solutions thus obtained *N*/10 HCl was added in amount equivalent to the NaOH employed. Concentrated HCl (2 cc.) was then added, 0·5 cc. of the solution of dimethyl-*p*-phenylenediamine sulphate, and finally two drops of ferric chloride solution. Estimations of the methylene blue so obtained were made in the extinctions apparatus (Table IV). The first two solutions were colourless.

Table IV.

SH in solution (mg.)	H ₂ S produced (mg.)	SH in solution (mg.)	H ₂ S produced (mg.)
1·52	0	3·04	0·24
1·88	0	4·86	0·60
2·43	0·14	5·25	0·63

The production of H₂S appears to require a certain minimum of SH before it becomes evident. The remaining figures are few, but if plotted they show a linear relation and suggest that 1 mg. SH after 2 hours' contact of the glutathione with sulphur yields 0·2 mg. H₂S.

THE RELATION BETWEEN SH-CONTENT OF TISSUES AND H₂S PRODUCTION WITH SULPHUR.

The literature contains a large number of estimations of the SH content of tissues. It would take, however, too much space to summarise them here. The following figures were obtained by me in the course of this research, using Tunncliffe's method [1925]. They are average values and are given in terms not of glutathione, but of SH (mg. per 100 g. tissue).

Liver: (cow) 36·4; (dog) 52·5; (rabbit) 45·1.

Kidney: (dog) 24·5; (rabbit) 21·6.

Thymus: (calf) 35·2.

Lung: (cow) 17·2; (dog) 9·5.

For the estimation of the yield of H₂S by tissues in contact with sulphur the same technique was employed as that described above. Under the circumstances of these experiments the tissues without added sulphur yielded no H₂S (Tables V and VI).

Liver was chiefly employed. In the case of smaller animals this was the only organ yielding enough material for a series of observations, and it easily yields uniform preparations. The degree of subdivision of a tissue is not without

effect upon the rate of H_2S formation. All values given represent the mean of two determinations; if these differed by more than 10 %, the result was rejected; the difference was usually much smaller.

The urethane narcosis consisted in the injection intraperitoneally of a 25 % solution of urethane in water, 4 g. per kg.

The relation between amount of tissue (or amount of SH contained in it) and H_2S evolved is in all cases strictly linear.

Table V.

Exp. no.	Weight of tissue (g.)	SH content (mg.)	H_2S evolved (mg.)
1	Liver	0.5	—
		1.0	—
		1.5	—
		2.0	—
2	Liver	0.5	—
		1.0	—
		1.5	—
		2.0	—
3	Liver	0.5	1.16
		1.0	2.32
		1.5	3.48
		2.0	4.64
4	Kidney	0.5	1.03
		1.0	2.06
		1.5	3.09
		2.0	4.12
5	Liver	0.5	0.88
		1.0	1.76
		1.5	2.64
		2.0	3.52

Further experiments were carried out upon various organs from different animals in order to determine the yield of H_2S from the existing concentration of SH in the tissue in question (Table VI).

If in these experiments only the amount of SH and the quantity of H_2S produced be considered, it will be evident that no parallelism or regularity is shown. This is in agreement with the statements of Hausmann.

In order to compare organs concerning their production of H_2S , experiments were carried out with the organs of the same animal, yielding the results given in Table VII.

For the comparison of the H_2S production per 1 mg. SH of the different organs, the experiments in which the animal was not narcotised are the most reliable. These also show that the values are not in agreement (Table VII).

From the figures the conclusion may be drawn that organs from larger animals such as the cow, dog and pig produce less H_2S than organs from small animals.

Hopkins [1921, 1922, 1925] finds in the tissues of small animals in all circumstances a higher reducing power than in the tissues of larger animals.

These experiments show that a marked difference exists between the H_2S formation by tissues and by reduced glutathione. The action of microbes can be excluded in the first case, for toluene was added and the period was too short. The rate of H_2S production was measured by starting a series of equal

Table VI.

LUNG.			
Exp. no.	mg. SH	mg. H ₂ S	Remarks
2a	1.20	0.40	Cat, organ fresh, no narcosis
3	1.23	0.48	Rabbit, organ 1 day old, 1 hour ether narcosis
4	1.60	0.87	Rabbit, organ 1 day old, 1 hour ether narcosis
5	1.64	0.60	Rabbit, organ 1 day old, urethane narcosis
6	1.79	0.95	Rabbit, organ 1 day old, urethane narcosis
7	2.49	0.12	Dog, organ 1 day old, 2 hours' ether-chloroform narcosis
8	2.75	1.11	Rabbit, organ 1 day old, 1 hour ether narcosis
9	3.32	0.15	Dog, same as in exp. 7
10	3.50	0.68	Rabbit, organ fresh, no narcosis
11	7.6	0	Cow, organ 1 day old
12	9.5	0	Cow, same as in exp. 11
13	12.62	0.28	Cow, organ 2 days old
LIVER.			
1	1.59	0	Rabbit, organ 1 day old, 1 hour ether narcosis
1a	1.64	1.35	Cat, organ fresh, no narcosis
2	3.17	0.69	Rabbit, organ 1 day old, urethane narcosis
3	3.19	0.64	Rabbit, organ 1 day old, 1 hour ether narcosis
4	3.21	0.28	Rabbit, organ 1 day old, ether narcosis
5	4.23	0.82	Rabbit, organ fresh, no narcosis
6	5.9	1.15	Rabbit, organ 1 day old, narcosis?
7	6.38	0.61	Rabbit, organ 1 day old, 1 hour ether narcosis
8	7.44	0.27	Dog, organ 1 day old, ether-chloroform narcosis
9	7.9	1.39	Rabbit, organ 1 day old, narcosis?
10	9.9	1.46	Rabbit, organ 1 day old, narcosis?
KIDNEY.			
1	1.02	0	Rabbit, organ 1 day old, urethane narcosis
2	1.34	0.73	Rabbit, organ 1 day old, 1 hour ether narcosis
3	1.51	0.72	Rabbit, organ 1 day old, 1 hour ether narcosis
4	1.61	0	Cow, organ fresh
5	1.65	0.50	Rabbit, organ 1 day old, ether narcosis
5a	1.70	0.71	Cat, organ fresh, no narcosis
6	2.18	0.02	Dog, organ 1 day old, ether-chloroform narcosis
7	2.19	1.07	Rabbit, organ fresh, no narcosis
8	2.20	0.54	Dog, organ 1 day old, 4 hours' ether-chloroform narcosis
9	2.48	0.26	Dog, organ 1 day old, ½ hour chloroform narcosis
10	2.72	0.22	Dog, organ 1 day old, 4 hours' ether-chloroform narcosis
11	3.16	0	Pig, organ 3 days old
12	3.30	0.05	Pig, organ fresh
13	3.70	0.83	Dog, organ 1 day old
14	4.26	0.29	Cow, organ 2 days old
15	5	0.43	Pig, organ 2 days old
16	6.57	2.53	Pig, organ 1 day old
THYMUS.			
1	1.19	0.25	Calf, organ 1 day old
2	2.39	0.45	Calf, organ fresh
3	3.30	0.39	Calf, organ 5 days old
4	12.82	1.37	Calf, organ 1 day old
5	13.06	1.10	Calf, organ 1 day old
SPLEEN.			
1	2.12	0.13	Cow, fresh
2	4.52	0	Cow, organ 1 day old
3	8.07	0	Cow, organ 2 days old
4	11.30	0	Cow, organ 1 day old
5	1.50	0	Rabbit, fresh, no narcosis
6	1.99	0.18	Cat, fresh, no narcosis

Table VII.

Exp. no.	Organ	mg. SH	mg. H ₂ S	mg. H ₂ S to 1 mg. SH	Remarks
1	Kidney	1.34	0.73	0.54	Rabbit, 1 hour ether narcosis
	Lung	1.23	0.48	0.39	
2	Liver	3.17	0.60	0.19	Rabbit, urethane narcosis
	Kidney	1.57	0.13	0.082	
	Lung	1.79	0.95	0.52	
3	Liver	1.59	0	0	Rabbit, 1 hour ether narcosis
	Liver	6.38	0.61	0.095	
	Kidney	1.51	0.72	0.47	
	Lung	1.60	0.87	0.54	
	Lung	0.80	0	0	
4	Liver	3.21	0.28	0.087	
	Kidney	1.65	0.50	0.303	
	Lung	1.15	0.09	0.078	
5	Brain	5.14	0.90	0.175	Rabbit, no narcosis
	Liver	4.32	0.82	0.189	
	Lung	3.50	0.68	0.194	
	Kidney	2.19	1.07	0.488	
	Spleen (1.5 g.)				
6	Liver	1.64	1.35	0.823	Cat, no narcosis
	Kidney	1.70	0.71	0.419	
	Lung	1.20	0.40	0.333	

quantities of tissues and measuring the amount of H₂S produced after a certain time. The curves of Fig. 4 show some examples: in the first hour or the first half hour much H₂S was produced, and the longer the period the less was produced.

Fig. 4. Rate of production of H₂S by liver.

- I Liver (cat) 1.56 mg. SH per 1 g.
 II Liver (cat) 1.83 "
 III Liver (dog) 3.58 "
 IV Liver (cat) 1.16 "
 V Liver (cat).
 VI Liver (cat) diluted.

Various factors which might throw light on the nature of the action were now investigated.

Temperature.

The tissues were treated in the usual way by rasping or cutting. The quantities were weighed and the bottles were placed in the boiling water-bath. The temperature in the bottles averaged between 70 and 80°. The amount of SH was also determined in heated and non-heated tissue by Tunncliffe's method. The coarseness of the tissue is important for the decrease in SH. The duration of heating also is of influence. The tissue used for H₂S production must always be of equal fineness (Table VIII).

Table VIII.

	mg. SH	mg. H ₂ S
1 g. liver, not heated	4.15	2.10
10 min. at 70–80°	3.05	1.10
2 g. liver mixture, not heated	2.9	0.69
5 min. at 70–80°	2	0.45
10 min. at 70–80°	0.70	0

In an SH solution obtained by treating tissue with trichloroacetic acid the amount of SH decreased in the same way in proportion to duration of heating and to temperature (Table IX).

Table IX.

	cc. N/100 Iodine
5 cc. SH solution, determined immediately	0.95
after 5 min. at 70–80°	0.83
after 10 min. at 100°	0.71

The H₂S production therefore is decreased by heating and dissolving, because the SH group is decomposed. This can be demonstrated in a simple way, by bringing together the solution, or the tissue rasped and diluted with a buffer solution, and sulphur in a bottle and introducing a lead acetate paper. The bottle is evacuated and placed in boiling water. The longer the bottle remains in water, the more H₂S is produced, and the SH content of the tissue or solution decreases. In one case 3 cc. SH solution contained 2.76 mg. SH when not heated; after boiling for 8 minutes, 2.16 mg.; in another case the non-heated SH solution contained 1.2 mg. SH; after boiling for 8 minutes it contained 0.75 mg. The same is true for tissues. When 1 and 2.1 cc. of tissue with lead paper are placed in boiling water for 1 and 2 minutes copious production of H₂S is noticed. When the same quantities without lead paper are heated for the same length of time and the lead acetate paper afterwards introduced, and the bottles then evacuated and placed again in boiling water for 1 and 2 minutes, H₂S production is still evident in the first case, but not in the second. The SH had therefore been decomposed by heating. Abelous and Ribaut also observed a temporary increase of H₂S production on heating.

Tissue yields H_2S on boiling only when S is added.

The rates of decrease of SH groups and of H_2S production are not proportional, as the H_2S production decreases much more rapidly. This agrees with the observation of Tsubura [1925] and Meyerhof [1926, 1928].

Influence of p_{H} .

In my experiments I used buffer solutions of primary and secondary phosphate (Clark). Equal quantities of tissue were brought together with 1 cc. of a phosphate buffer solution (Clark).

Some of the results obtained are shown in Fig. 5. The absolute heights of the curves are not comparable, but this is unnecessary since, as remarked already, the duration of the experiments varied. From these curves may be deduced that generally H_2S production does not take place in a system at p_{H} 4. With increasing alkalinity H_2S production starts or increases and reaches its maximum at p_{H} 7-8. If the alkalinity increases still more the production decreases again. No error is caused by absorption of H_2S by the buffer.

Fig. 5. Influence of p_{H} on H_2S production of tissues.

2 Liver	0.61 mg. SH.
3 Liver	0.61 "
4 Kidney	0.845 "
6 Kidney	1.66 "
7 Kidney.	
10 Liver.	

Influence of H-donators.

Concerning glutathione, Hopkins [1922] states that neither succinate nor citrate accelerates the reduction of glutathione. Reduced glutathione does not act as a H-donator in Thunberg's sense. This is deduced by Hopkins from equilibrium determinations in which no relation could be found between the quantity of methylene blue reduced and the quantity of H-donator changed.

In preliminary experiments I used a 1 % solution of potassium succinate and observed that this caused the H₂S production in various organs to increase (Table X).

Table X.

	mg. SH to 1 g. tissue	mg. H ₂ S produced
4 g. lung tissue	3.32	0.15
+ 1 cc. 1 % K ₂ succinate	3.32	0.51
1 g. liver	3.17	0.69
+ 1 cc. 1 % K succinate	3.17	1.13
1 g. liver	—	1.65
+ 1 cc. 1 % K succinate	—	1.73
1 cc. kidney mixture	0.51	0
+ 1 cc. 1 % K succinate	0.51	0.27
2 g. kidney	—	0.29
+ 1 cc. 1 % K succinate	—	0.44
1 g. lung	1.64	0.58
+ 1 cc. 1 % K succinate	1.64	0.02
2 g. kidney	—	0.29
+ 1 cc. 1 % K succinate	—	0.47
5 g. spleen	11.30	0
+ 1 cc. 1 % K succinate	11.30	0.23

Those preliminary experiments therefore show, contrary to the results of Hopkins obtained with glutathione, that H₂S production is influenced by hydrogen donors. If the tissue alone produces a relatively large amount of H₂S the effect of adding a hydrogen donor is smaller than in a case in which the H₂S production as such is slight. The experiments cannot be compared quantitatively since the times were not the same.

It is possible that the greater or less effect of the added hydrogen donor is dependent on the amount of natural hydrogen donor contained in the tissue at the moment of the experiment.

In addition to succinate, tartrate, citrate and malate were also investigated. Thunberg [1918, 1920] obtained with these salts an acceleration of the decoloration of methylene blue and drew the conclusion that they might act as hydrogen donors. The solutions used by me were 0.1 *N* accurately neutralised to phenol red at *p*_H 7.6. To the tissue 1 cc. of the donor was added.

The quantity of H₂S produced was compared with the quantity produced by tissue + 1 cc. phosphate buffer at *p*_H 7.6 or with 1 cc. water at *p*_H 7.6 (mixture of tap water at *p*_H 7.8 and distilled water at *p*_H 6.2) (Table XI).

Table XI.

	mg. H ₂ S					
1 cc. <i>N</i> /10 tartrate + tissue	0.215	—	—	0.41	—	0.97
1 cc. <i>N</i> /10 citrate + tissue	0.79	0.70	0.77	1.07	0.90	2.42
1 cc. <i>N</i> /10 malate + tissue	0.19	—	0.32	0.45	0.32	0.91
1 cc. <i>N</i> /10 succinate + tissue	0.21	0.36	0.36	—	—	0.835
1 g. liver + 1 cc. buffer <i>p</i> _H 7.6	0	0	0	0.37	0.13	—
1 g. kidney + 1 cc. buffer <i>p</i> _H 7.6	—	—	—	—	—	0.68

Tartrate, malate and succinate have apparently an equal ability to act as hydrogen donor, but citrate yields without exception a much larger H₂S production. Controls without tissue gave negative results.

If only the hydrogen directly combined with C acts as a donator, as Thunberg assumes, the larger number of H atoms (4 in citrate and succinate, 2 and 3 in the tartrate and malate) cannot be the cause. But it is possible that the H of the OH group in citric acid is more mobile than the H in the OH group in malic and tartaric acids. It is remarkable that Tsubura was not able to demonstrate this action of citrate, but observed a renewed power of reduction in washed muscle pulp after adding potassium succinate.

The few experiments carried out by me with reduced glutathione and hydrogen donators also showed an increased H_2S production (Table XII).

Table XII.

	mg. SH
Glutathione + buffer solution (p_H 7.6) + S	0.04
+ citrate $N/10$ + S	0.18
+ buffer solution (p_H 7.6) + S	0.35
+ succinate $N/10$ + S	0.42

In order to investigate the influence of the concentration of the hydrogen donator various dilutions of citrate and malate were used.

$M/10$ citric acid (21.01 mg. to 1 cc.)

$M/10$ malic acid (13.4 „ „)

From both solutions 5 dilutions were prepared, from the citrate 10.5, 5.25, 2.625, 1.31 and 0.65 mg., from the malate 6.7, 3.3, 1.65, 0.82 and 0.41 mg. in 1 cc.

Fig. 6. Effect of malate as H-donator.

The curves (Figs. 6 and 7) show the H_2S production in these concentrations with different tissues. In the first place the steeper course of the curves after adding citrate is remarkable, *i.e.* the increased H_2S production in comparison with that after adding malate.

In the second place it is evident that, if the tissue as such has produced much H_2S , adding a small amount of donator has little or no influence.

Thirdly, both donators, but citrate more clearly than malate, show a decrease in H_2S production when the donator concentration is increased. The curve is parabolic. The optimum activity is situated at $6/20 M$ to $1/10 M$ for both salts.

A single experiment was made with citrate at higher concentration, which showed that the largest amount of H₂S was liberated at such a concentration (Table XIII).

An inhibition of H₂S production by adding donator was never observed if the p_H were carefully adjusted.

Table XIII.

	mg. citrate	mg. SH	mg. H ₂ S
1 g. liver + 1 cc. buffer p_H 7.6	0	1.51	0.60
liver + citrate $M/2$	100.5	—	1.23
liver + citrate diluted $\times 2$	50.25	—	1.58
liver + citrate diluted $\times 4$	25.15	—	1.72
liver + citrate diluted $\times 8$	12.6	—	1.80

Influence of dehydrogenase.

In order to investigate the influence of dehydrogenase on the H₂S production of tissues a solution was prepared by Ohlson's method [1921]. 0.5 to 1 kg. of lean horseflesh, freed from fat and fascia, was ground three times and washed with 0.25 % NaCl solution until colourless, filtered through a cloth and pressed. The residue was shaken for an hour with an equal volume of 2.4 % Na₂HPO₄ solution and centrifuged.

Fig. 7. Effect of citrate as H-donor.

The enzyme solution had a much lower activity in presence of sulphur than tissue and also gave a much fainter nitroprusside reaction. In these experiments I used Thunberg bottles in which a lead acetate paper was placed, the bottles being left in the thermostat for 20 hours. These experiments were not quantitative but the difference in hue of the lead papers afforded an approximate indication.

1. *Temperature.* Heating at 65° for 10 minutes has little influence (in spite of the fact that according to Thunberg all dehydrogenases are inactivated at 60°). If the tubes be placed in boiling water after evacuation and introduction of the lead acetate paper, the papers are immediately coloured black; as in the case of tissue boiling causes much H₂S to be produced quickly, the quantity of SH is exhausted and therefore after boiling but little H₂S can be produced.

2. *Effect of p_H .* A marked H_2S production was observed at p_H 5, sometimes larger, sometimes smaller, than at p_H 7.6. At p_H 8.3 the H_2S production is positive but less marked.

3. *H-donators.* As is the case of the tissue experiments addition of citrate promotes H_2S production. Succinate also promotes H_2S production. The effect of adding hydrogen donators is only evident if the enzyme solution be not too concentrated.

Those experiments show that the enzyme (dehydrogenase) solution used had the same activities as tissue or glutathione but to a much less pronounced extent.

If the enzyme solution be added to normal tissue, little increase in H_2S production can be noticed in 1 to $1\frac{1}{2}$ hours.

SUMMARY.

1. Three different sources of SH have been used.

(1) Tissues.

(2) SH solutions (glutathione or organ extracts).

(3) A dehydrogenase solution.

All these sources yield H_2S after addition of S. In reduced glutathione, as well as in tissue, H_2S production is during a certain time directly proportional to the quantity of SH present. Since all tissues contain SH, the H_2S production can be explained undoubtedly by the purely chemical process:

The H_2S production by tissues is much more marked than by the pure SH compound, when both contain the same amount of SH. This is not necessarily due to enzyme activity, but can be explained by the possibility that the SH group in tissues is more active than in the pure compound. Catalytic factors (iron catalysis, as mentioned by Warburg and Sakuma) may play a more important part in the tissues than in the pure SH preparation.

A solution of dehydrogenase (an enzyme which may be expected to yield H_2S when brought together with S) produces very little H_2S . If the enzyme be added to normal tissue no increase in H_2S production results. It is a remarkable fact that the organs of small animals have a higher ability to produce H_2S than those of large animals, the amount of SH being the same.

2. With all three sources of SH the influence of various factors was investigated.

(1) *Temperature.* Increase of temperature, even up to boiling point, results in promotion of H_2S formation and decrease of the SH content of tissue. This phenomenon cannot be explained by enzyme activity; on the contrary, an enzyme should be destroyed at 100° . If the H_2S production of heated tissue is determined, it appears that the H_2S production decreases more quickly than the SH content. The heating therefore probably affects the other factors that are concerned with H_2S production.

(2) p_H . In a strongly acid or alkaline system the H₂S production is small or nil. The optimum is at about the neutral point. This holds for glutathione as well as for tissues and enzyme solutions.

(3) *H-donators*. Addition of H-donators results in an increase of H₂S production. If the tissue as such is able to produce a large amount of H₂S with S, the addition of H-donators is of little effect. Citrate gives rise in all three cases to the largest increase in H₂S production. The donators have an optimum concentration between $M/10$ and $M/20$.

3. Since H₂S production may take place under circumstances in which enzymes are inactive and since an enzyme solution does not produce more H₂S than corresponds with its content of SH, I believe the conclusion to be justified that H₂S production in living tissue after addition of sulphur is not due to enzyme activity.

REFERENCES.

- Abelous and Ribaut (1903). *Compt. Rend. Acad. Sci.* **137**, 95, 268.
De Rey Pailhade (1888). *Compt. Rend. Acad. Sci.* **106**, 1683.
— (1894). *Compt. Rend. Soc. Biol.* **1**, 455.
Heffter and Hausmann (1904). *Hofmeister's Beitr.* **5**, 213.
— (1908). *Med. Naturwiss. Arch.* **1**, 81.
Hopkins (1921). *Biochem. J.* **15**, 286.
— (1922). *J. Biol. Chem.* **54**, 527.
— (1925). *Biochem. J.* **19**, 787.
Meyerhof (1926). *Pflüger's Arch.* **170**, 367.
— (1928). *Pflüger's Arch.* **199**, 531.
Ohlson (1921). *Skand. Arch. Physiol.* **41**, 77.
Strassner (1910). *Biochem. Z.* **29**, 295.
Thunberg (1918). *Skand. Arch. Physiol.* **35**, 163.
— (1920). *Skand. Arch. Physiol.* **40**, 1.
Tsubura (1925). *Biochem. J.* **19**, 397.
Tunncliffe (1925). *Biochem. J.* **19**, 194, 199.