STUDIES ON BLOOD CHANGES IN PNEUMOCOCCUS INFECTIONS.

AN EXPERIMENTAL STUDY OF THE FORMATION AND FATE OF METHEMOGLOBIN IN THE BLOOD.

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(Received for publication, January 27, 1921.)

INTRODUCTION.

Methemoglobin Formation in Pneumonia.

The pneumococcus, both *in vitro* (Gilbert and Fournier, 1896; Grüter, 1909; and Peabody, 1913) and *in vivo* (Butterfield and Peabody, 1913), has been shown to transform hemoglobin into methemoglobin. In consequence it seemed that methemoglobin formation might be the chief cause of the cyanosis sometimes observed in pneumonia. A study of the oxygen content and capacity of venous and arterial blood, however (Stadie, 1919), showed that the great and constant abnormality accompanying cyanosis was an increased proportion of reduced hemoglobin to oxyhemoglobin in the arterial blood. Therefore the essential cause of the cyanosis of pneumonia is incomplete oxygenation of the arterial blood, rather than the presence of methemoglobin in the blood. In only one of the thirty-two cases studied by the author was the oxygen capacity significantly reduced, as it would be if any considerable portion of the hemoglobin were changed to methemoglobin. On the contrary, in many cases the oxygen capacity of the blood was above the normal average.

Although we had but one case in which the oxygen capacity fell significantly, Peabody (1913) and Harrop (1919) have observed several cases in which such a fall occurred. Of eleven cases Peabody observed a marked fall of oxygen capacity in three, and Harrop in nine cases found a decrease of one-half in the total oxygen capacity in two. All these cases had positive blood cultures. In two other cases of Harrop's with negative blood cultures there was a decrease in oxygen capacity, but of less degree and over a greater period of time. It is possible that in these cases a considerable proportion of the hemoglobin may be altered into methemoglobin, and even in patients that do not show definitely lowered oxygen capacity small amounts of methemoglobin may be formed, and either be eliminated from the blood, or remain in it. In order to obtain more complete

evidence on these points a quantitative method for determination of methemoglobin was devised (Stadie, 1920), and in the present study it has been utilized in experimental work aimed to obtain evidence on the points in question.

General Conditions for Methemoglobin Formation.

The agents which change hemoglobin to methemoglobin are of varied nature. They include the following groups of known chemical substances: oxidizing substances, ozone, iodine, chlorates, ferricyanides, nitrites, nitrates, and azo compounds; reducing substances, pyrogallol, hydroquinone, hydroxylamine, etc.; organic bases, aniline, phenacetin, acetanilide, and toluidine; salts, sodium chloride in concentration above 1.5 per cent and ammonium sulfate in saturated solution. They also include several races of bacteria: *Streptococcus viridans*, cholera, pneumococcus, Gaertner bacillus, and certain nitrosobacilli (Wallis, 1913–14).

In vitro all these agencies produce methemoglobin with greater or less facility. Potassium ferricyanide rapidly forms methemoglobin from oxyhemoglobin. An amount of oxygen is liberated which is equivalent to the dissociable oxygen originally combined as oxyhemoglobin. Sodium nitrite converts hemoglobin to methemoglobin *in vitro*, but more slowly than potassium ferricyanide. The nitrite liberates an amount of oxygen equivalent to the amount necessary to change the nitrite to nitrate (Barcroft and Müller, 1911-12). In other words, two molecules of nitrite transform one molecule of hemoglobin, producing one molecule of oxygen and one molecule of methemoglobin. Hydroxylamine transforms hemoglobin quantitatively, molecule for molecule (Letsche, 1912).

Of the above agents, we have used in our experiments potassium ferricyanide and sodium nitrite.

Characteristics of Clinical and Experimental Methemoglobinemia.

Agents.—Human cases of methemoglobinemia are the result of poisoning with various agents, of which aniline is perhaps the most familiar. In laboratory animals methemoglobin is produced easily by nitrites, potassium ferricyanide, acetanilide, and the pneumococcus.

Symptoms.—In the overwhelming methemoglobinemia produced experimentally it is difficult to dissociate the symptoms produced by

the drug from those produced by the methemoglobin. Rapid breathing and air-hunger are constant symptoms in severe grades of methemoglobinemia, and when 70 to 80 per cent of the hemoglobin is changed the animal exhibits all the signs of acute suffocation and dies in a short time. In milder instances with a destruction of 25 to 50 per cent of the hemoglobin, usually there are no symptoms in rabbits. It is doubtful whether the mere presence of large amounts of methemoglobin in the body is harmful.

Cyanosis is a regular accompaniment of methemoglobinemia. This, of course, is due to the fact that the presence of methemoglobin in blood gives it a dark color closely resembling that of venous blood. Transformation of as little as 5 per cent of the hemoglobin to methemoglobin in blood gives it a dark color. The blood does not become bright red on exposure to the air and is easily distinguishable from normal oxygenated blood. The cyanosis itself is indistinguishable from the cyanosis due to oxygen unsaturation of the arterial blood. Two important differences, however, may be noted: (1) the cyanosis of oxygen unsaturation usually accompanies pulmonary or heart disease and varies in intensity with change of position, coughing, and exertion; (2) the administration of oxygen may diminish this cyanosis. The final differentiation is made by the spectroscope or by methemoglobin determination, for which a method has been recently published (Stadie, 1920).

Pathology.—No characteristic changes of the organs have been described in animals in which a severe methemoglobinemia has been induced. Certain hemorrhagic changes and areas of necrosis in the liver and spleen have been described, but these have been inconstant and have followed large doses of potassium chlorate, and consequently might easily have been due to this substance rather than to the methemoglobin.

The proportion of hemoglobin changed may be as great as 100 per cent, as after the intravenous injection of sodium nitrite in rabbits. With acetanilide or sodium nitrite in proper doses a reduction in the amount of hemoglobin of 60 to 70 per cent may be brought about easily. The animals often recover; the sudden flooding of the body by so large an amount of methemoglobin is without apparent permanent effect.

Methemoglobin may exist in the blood in two distinct conditions. First, it may be present in the plasma alone, a true methemoglobinemia. This is, however, rare, but is illustrated by Brandenburg's case of potassium chlorate poisoning which showed a rapid decrease of red blood cells from 4,300,000 to 1,600,000 in 6 days. The serum showed methemoglobin spectroscopically. Second, the methemoglobin is present within the red blood cells—a condition of methemoglobincythemia. This is the usual occurrence. Cases of nitrite, acetanilide, and nitrobenzene poisoning and bacterial infections with methemoglobin formers fall into this class.

EXPERIMENTAL.

Fate of Methemoglobin.—Methemoglobin solutions injected intravenously are rapidly eliminated from the blood. Table I shows

TABLE I.

Elimination of Methemoglobin Following Intravenous Injection in Rabbits.

Rabbit No.	Time.	Injection.	Hemoglobin per 100 cc. of blood.	Methemoglobin spectrum.		
				Serum.	Cells.	Urine.
	p.m.		*gm.*			
1	12.10		13.87			
	12.20	2 gm. of methemoglobin intravenously.				
	12.45		12.04	Negative.	Negative.	
	4.00	(Killed.)*	11.87	"	"	++++
	a.m.					
2	9.05		10.03			
	11.00	1.7 gm. of methemoglobin intravenously.				
	11.15		10.03	Negative.	Negative.	++++

* Extracts of lungs, liver, spleen, heart, feces, and intestines showed no methemoglobin spectroscopically.

the extreme rapidity with which methemoglobin dissolved in the plasma disappears. The hemoglobin was determined gasometrically by Van Slyke's method (1918), the methemoglobin by the author's method (1920).

Within 15 to 25 minutes an amount of methemoglobin equal to 20 to 22 per cent of the total hemoglobin was completely removed from

the blood so that none could be found in it spectroscopically. At least part of the methemoglobin was excreted in the urine.

Storage of Methemoglobin.—Extracts of the various organs made immediately post mortem revealed no evidence that methemoglobin is accumulated in any one place. When the methemoglobin is present in the red blood cells only, even when all the hemoglobin is changed to methemoglobin, with death by virtual suffocation due to lack of labile oxygen, the plasma is always free from methemoglobin bands.

The methemoglobin produced in the red blood cells is rapidly destroyed. In the milder cases of experimental methemoglobincythemia this destruction was found to be so rapid that repeated and careful examinations of blood cells, plasma, urine, and tissues failed to reveal its presence.

Table II gives the results from rabbits injected with solutions of potassium ferricyanide. The action of this substance *in vivo* is relatively slow, but by repeated injections over a period of several hours almost 50 per cent of the hemoglobin may be changed. In the three rabbits the potassium ferricyanide changed 43, 30, and 19 per cent respectively of the hemoglobin, doubtless into methemoglobin, and yet the latter was so rapidly destroyed that practically none of it could be found in the blood.

In another rabbit (Table III) within 5 days the hemoglobin diminished 39 per cent, yet no methemoglobin was found spectroscopically in either plasma or cells. Moreover, the urine and the aqueous extracts of liver, lungs, spleen, kidney, heart, skeletal muscle, intestines, and feces showed no methemoglobin by spectroscope.

The rapid production of methemoglobin, as by the injection of sodium nitrite, by which an easily controllable degree of methemoglobin formation may be brought about, gives the same results.

In Rabbit 7 (Table IV) practically all the hemoglobin was changed to methemoglobin, the animal dying immediately of suffocation. The pigment is wholly in the cells. Rabbit 8 lived for 26 minutes with hemoglobin of only 2.9 gm. per 100 cc. of blood, or 23 per cent of the initial value. Yet within this short time the methemoglobin had decreased to 6.7 gm. per 100 cc. of blood. A rabbit of 2.85 kilos has 142 cc. of blood (5 per cent by weight (Van Slyke and Salvesen, 1919)), or in this case 18.1 gm. of hemoglobin, of which at death 4.1

TABLE II.

Production of Methemoglobin in Rabbits by Potassium Ferricyanide.

Rabbit No.	Date.	Time.	Injection.	Hemoglobin per 100 cc. of blood.	Methemoglobin per 100 cc. of blood.
	1919	*a.m.*		*gm.*	*gm.*
3	Apr. 25	9.30		15.2	
		10.15	10 cc. of 0.02 M potassium ferricyanide intravenously.		
		10.30		13.2	
		11.50	10 cc. of 0.02 M potassium ferricyanide intravenously.		
		p.m.			
		12.15		11.0	
		2.45	5 cc. of 0.02 M potassium ferricyanide intravenously.		
		3.15		8.8	
	Apr. 29			8.9	
	May 6			8.7	0.0
		a.m.			
4	May 7	11.35		11.1	
		11.45	5 cc. of 0.02 M potassium ferricyanide intravenously.		
		p.m.			
		12.45		10.5	0.0
		1.02	5 cc. of 0.02 M potassium ferricyanide intravenously.		
		2.15		10.0	0.3
		2.30	5 cc. of 0.02 M potassium ferricyanide intravenously.		
		2.31	(Animal died.)	7.8	0.2
		a.m.			
5	May 9	9.20		13.3	
		9.40	20 cc. of 0.02 M potassium ferricyanide intravenously.		
		9.45		14.1	0.0
		10.30		13.0	0.4
		11.45		12.5	0.0
		p.m.			
		2.07	10 cc. of 0.02 M potassium ferricyanide intravenously.		
		2.30		10.8	0.0

TABLE III.

Production of Methemoglobin in Rabbits by Potassium Ferricyanide.

Rabbit No.	Date.	Time.	Injection.	Hemoglobin per 100 cc. of blood.	Methemoglobin per 100 cc. of blood.	Methemoglobin spectrum.	
						Plasma.	Cells.
	1919	*m.*		*gm.*	*gm.*		
6	June 5	12.00					
		p.m.					
		2.27	10 cc. of 0.02 M potassium ferricyanide intravenously.	11.6*			
		a.m.					
	June 6	10.00	5 " " 0.02 M " " "				
		11.07	5 " " 0.02 M " " "				
		11.47	5 " " 0.02 M " " "				
		p.m.					
		12.21	5 " " 0.02 M " " "				
		1.45	5 " " 0.02 M " " "				
		2.47	5 " " 0.02 M " " "				
		4.00	5 " " 0.02 M " " "				
		a.m.					
	June 7	10.58	5 " " 0.02 M " " "				
		11.45	5 " " 0.02 M " " "				
		p.m.					
		1.15	5 " " 0.02 M " " "	8.2	0.2		
	June 9	3.40	10 " " 0.02 M " " "				
		4.32	10 " " 0.02 M " " "				
	June 11	4.30	(Killed.)†	7.0	0.5	Negative.	Negative.

* Colorimetric determination.

† Extract of liver, lungs, spleen, heart, skeletal muscle, intestines, and feces showed no methemoglobin spectrum. Urine negative also.

gm. were left. Of the 14 gm. of methemoglobin formed, only 9.5 gm. remained at death. In other words, 4.4 gm. of methemoglobin were destroyed in 26 minutes. In Rabbit 9, 1.8 gm. of methemoglobin were destroyed in 30 minutes. In all these rabbits no methemoglobin was found in the plasma, although the blood was dark chocolate in color; it was present in the cells only.

Therefore even in extreme cases of methemoglobin production in which death results quickly from an insufficient oxygen supply, there is a rapid destruction of methemoglobin. When the methemoglobin is produced more slowly this destruction is fast enough to prevent its accumulating in the blood in sufficient quantity to be detected by the spectroscope. Not only is this true when chemicals are the causative agent, but also when methemoglobin-producing bacteria act *in vivo*.

However, when the production of methemoglobin is very extensive, *e. g.* 30 to 50 per cent of total pigment, and sudden, as following intravenous injections of sodium nitrite, methemoglobin may be found in the blood, but always in the red blood cells. Even in these instances, if the animal survives for a comparatively brief time, the methemoglobin disappears from the blood.

The mechanism of this disappearance has not been determined. Pearce, Austin, and Eisenbrey (1912) studied the fate of hemoglobin injected intravenously and found that no hemoglobin was eliminated through the kidney unless the rate of injection was above a minimum which was high, but that nevertheless the injected hemoglobin rapidly disappeared from the blood stream. Furthermore, Whipple and Hooper (1913) showed that injected hemoglobin is changed rapidly to bile pigments in normal dogs and in dogs with Eck fistula or hepatic ligation. Since methemoglobin is closely related chemically to hemoglobin it is possible that it is similarly disposed of.

Action of Pneumococci on Hemoglobin in Vivo.—Grüter (1909) inoculated a cat intraperitoneally with a large amount of pneumococcus culture but was unable to demonstrate methemoglobin in the blood. Peabody (1913) developed the subject further by inoculating rabbits intravenously with the organisms from 300 to 600 cc. of 24 hour broth cultures of pneumococci. Death resulted within a few hours and the blood always showed on direct film enormous num-

TABLE IV.

Intravenous Injection of Sodium Nitrite in Rabbits. Rapid Formation and Disappearance of Methemoglobin.

Rabbit. No.	Weight.	Time.	Injection.	Hemoglobin per 100 cc. of blood.	Methemoglobin per 100 cc. of blood.	Methemoglobin* destroyed.	Methemoglobin spectrum.		
							Plasma.	Cells.	Urine.
	kg.	*a.m.*		*gm.*	*gm.*	*gm.*			
7		9.15		7.8					
		9.30	0.95 gm. of sodium nitrite intravenously.						
		9.31	(Died.)	0.3			Negative.	++++	Negative.
		p.m.							
8	2.85	3.00		12.7	0.0				
		3.21	0.1 gm. of sodium nitrite per kilo intravenously.						
		3.47	(Died.)	2.9	6.7	4.4	Negative.	++++	Negative.
		a.m.							
9	1.7	9.00		10.0	0.0				
		9.30	0.12 gm. of sodium nitrite per kilo intravenously.						
		10.00	(Died.)	3.6	4.3	1.8	Negative.	++++	Negative.

* Estimated on the assumption that the blood volume is 5 cc. per 100 gm. of body weight (Van Slyke and Salvesen, 1919).

bers of pneumococci. With these overwhelming pneumococcemias Peabody showed that there was a rapid fall in total hemoglobin as measured by oxygen capacity, but he was rarely able to demonstrate the presence of methemoglobin in the blood. He nevertheless concluded that this fall in total oxygen capacity (hemoglobin) was due to a methemoglobin production. However, when these experiments are repeated with quantitative determination of methemoglobin, it is clear that bacterial methemoglobinemia is similar to that produced chemically in that the methemoglobin formed rapidly disappears.

Rabbits were inoculated by ear vein with the centrifuged pneumococci from 24 hour cultures resuspended in a small amount of saline solution. Death occurred in all cases and all showed by direct film of the blood enormous numbers of organisms. The results are shown in Table V.

In Rabbit 10, after 5 hours, despite a fall in hemoglobin of 3.94 gm. per 100 cc. of blood (30 per cent of the total), there was no methemoglobin in the blood. In No. 12, 3.82 gm. of hemoglobin per 100 cc. of blood (30 per cent of the total) were changed, but only 0.83 gm. of methemoglobin per 100 cc. was found. In Nos. 10 and 11 at death considerable amounts of methemoglobin were found in the blood, but amounts representing only 30 to 32 per cent of the total change. It is clear then that the mechanism here is the same that is described in the production of methemoglobin by chemicals. In the gradual change of hemoglobin to methemoglobin the latter pigment disappears as fast as it is formed, so that it is never present in the blood in sufficient concentration to be detected spectroscopically. Only near death does the rapid production of methemoglobin by the huge numbers of pneumococci lead to its accumulation in the blood in quantities greater than the now disordered metabolism can handle. In other words, it is rarely possible to find methemoglobin in the blood, unless just before death, even after considerable changes of hemoglobin to methemoglobin.

It is to be further noted that the methemoglobin present in these instances is entirely intracellular. Examination of the plasma spectroscopically gives negative results, but the centrifuged cells in the last samples of Nos. 11 and 12 contain abundant methemoglobin. The urine also is always negative. The condition is a methemoglobincythemia.

TABLE V.

Production of Methemoglobin by Pneumococci in Rabbits.

Rabbit No.	Date.	Time.	Conditions.	Hemoglobin per 100 cc. of blood.	Hemoglobin decrease per 100 cc. of blood.	Methemoglobin per 100 cc. of blood.	Remarks.
	1919	*a.m.*		*gm.*	*gm.*	*gm.*	
10	June 15	11.45		12.98	0.0	0.0	
		p.m.					
		1.00	Inoculated.				
		3.30		10.82	2.16	0.30	Blood bright red.
		5.00		9.04	3.94	0.00	" " "
		8.45	Very sick.	6.02	6.96	2.09	" chocolate-colored.
		9.00	Died.				
		a.m.					
11	June 25	9.00		12.78		0.0	
		10.25	Inoculated.				
	June 26	10.25		11.60	1.18	0.18	Blood bright red.
		p.m.					
		4.19	Died.	5.75	7.03	2.27	" chocolate-colored.
		a.m.					
12	June 30	9.00		12.87		0.0	
		10.50	Inoculated.				
		p.m.					
		3.30	Died.	9.05	3.82	0.83	Blood bright red.

Rabbit 10 received Pneumococci Type I from 250 cc. of broth cultures. Rabbit 11 received Pneumococci Type I from 100 cc. of broth cultures. Rabbit 12 received Pneumococci Type II from 150 cc. of broth cultures. Postmortem films of blood from all the rabbits showed enormous numbers of pneumococci.

Non-Production of Methemoglobin by Pneumococcus Autolysates.

Grüter (1909) observed methemoglobin formation in the presence of dead cultures, filtrates, and centrifugates. Butterfield and Peabody (1913) also have produced methemoglobin by the autolysates of pneumococcus cultures. Rieke (1904), however, attributed methemoglobin formation to the living pneumococcus and streptococcus only. Cole (1914) likewise reached the same conclusion, since he was unable to demonstrate the production of methemoglobin by filtrates and extracts of pneumococcus cultures.

We have also been unable to show a transformation of hemoglobin to methemoglobin without the living organism. A broth culture of pneumococcus was used in which, after 5 days incubation, all organisms were autolyzed as shown by sterile subcultures. Prepared in this way the pneumococcus autolysate failed to produce methemoglobin when mixed with hemoglobin solutions, as shown in Table VI.

It is quite possible, however, that if, as has been suggested by Avery and Cullen,[1] the proper conditions are found for the reaction or if concentrated solutions of intracellular products obtained by the disintegration of the pneumococcus are used, methemoglobin formation without the living organism may be demonstrated.

TABLE VI.

Action of Autolysate of Pneumococci on Hemoglobin.

Tube No.	Conditions.	Broth.	Autolysate.	Hemoglobin solution.	Initial hemoglobin.	Hemoglobin after 20 hrs.	Methemoglobin after 20 hrs.
		cc.	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*
1	Room temperature.....	0	10	10	7.14	6.45	0.69
2	Ice box.....	0	10	10	7.14	7.14	0.00
3	Room temperature.....	10		10	7.30	6.67	0.63

DISCUSSION.

Methemoglobin Formation in Pneumonia.—Peabody (1913) was the first to explain fall of blood oxygen capacity observed in some (usually fatal) pneumonia cases as due to a change of hemoglobin to methemoglobin. He suggested that this alteration could take place through the action of a soluble product of bacterial metabolism. If the production of methemoglobin by soluble bacterial products, as suggested by Peabody, is substantiated by further experiments, then it is possible that the decrease in hemoglobin may be brought about by the escape of these products into the blood stream from a focus of pneumococcic infection in the lungs. However, all the cases studied by Peabody (1913) and Harrop (1919) with a sudden and well defined decrease of hemoglobin had a pneumococcemia of profound degree (from 2,500 to 16,000 colonies per cc. of blood). It is, therefore,

[1] Avery, O. T., and Cullen, G. E., unpublished work.

probable that a great production of methemoglobin occurs only when the opportunity for the direct action of the pneumococci on the blood is greatest; *i. e.*, in cases with a bacteremia.

Methemoglobin and Cyanosis in Pneumonia.—Although there has been a prevalent belief that methemoglobinemia is a factor in the cyanosis of pneumonia, many observers (Abrahams, Hallows, and French, 1919; Synnott and Clark, 1918; and Peabody, 1913) have looked for it spectroscopically in cases of pneumonia deeply cyanotic, but have failed to find it.

The absence of methemoglobinemia in pneumonia, even when there is a marked fall in oxygen capacity, is, we believe, explainable by the results reported in this paper. Methemoglobin disappears from the circulation with great rapidity, whether it is introduced by injection of methemoglobin or formed within the circulation by the action of chemicals or of pneumococci. If we take into consideration this fact, the ability of the pneumococcus to form methemoglobin, and the consistent failure to find it in the blood in the cyanosis of pneumonia, it appears that the following conclusions represent the most probable explanation of what occurs.

CONCLUSIONS.

In the occasional cases of pneumonia which show a decrease in the oxygen capacity of the blood, the decrease is probably due to a formation of methemoglobin. The latter is removed from the circulation, however, as rapidly as it is formed, so that it can seldom be detected even qualitatively, and is probably never a cause of cyanosis.

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