77. THE OXIDATION OF SULPHANILAMIDE AND SULPHAPYRIDINE BY HYDROGEN PEROXIDE

By G. V. JAMES¹

From the Bernhard Baron Research Laboratories, Queen Charlotte's Hospital, Hammersmith, W. 6

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A PURPLE pigment was isolated from the urine of patients treated with sulphanilamide and from the urine of two patients who had received sulphapyridine. Since the pigments were probably oxidation products of the drugs, the following chemical oxidation experiments were undertaken to determine if the *in vitro* changes resembled those occurring *in vivo*.

Colebrook & Purdie [1937] diagnosed cyanosis in 53 cases and detected methaemoglobin or sulphaemoglobin in 45 of them, leaving 8 patients in whom cyanosis might have been due to pigment formation from the drug.

Marshall & Walzl [1937] found methaemoglobin in only 2 of 7 cyanosed patients receiving sulphanilamide. These authors obtained a black oxidation product by the action of calcium hypochlorite on sulphanilamide and attempted unsuccessfully to isolate a pigment from dark-coloured bloods taken from cyanosed patients in whom methaemoglobin was absent.

Ottenberg & Fox [1938] were led by the helio-sensitivity of patients treated with sulphanilamide to try the effect of ultraviolet light on dilute sulphanilamide solutions; brief exposure to the light produced a strong violet colour accompanied by a 30–60% diminution of the sulphanilamide content of the solution. The violet colour was absorbed by red blood cells and the authors considered that the colour of the red cells of patients undergoing therapy might have a similar origin.

Harris & Michel [1939], in a survey of sulphanilamide cyanosis, observed in several samples a diffuse darkening of the blood with maximum absorption in the red at 670 m μ . Methaemoglobin and sulphaemoglobin were absent from these samples and the pigment faded in several hours even at 0°. The colour of the plasma was normal so the pigment was neither haematin nor methaemalbumin.

In the first experiments, hydrogen peroxide and calcium hypochlorite of equivalent oxygen availability were added to sulphanilamide solutions which were prepared in physiological saline, buffered at pH 7.4. Some of the solutions were left exposed to light at laboratory temperature (about 17°), others were kept in the dark at the same temperature and others were incubated in the dark at 38°. The solutions containing hydrogen peroxide in the dark at laboratory and at body temperatures did not lose sulphanilamide; the others did as shown in Table 1.

A red insoluble pigment was deposited from the solution containing peroxide. The solution treated with hypochlorite went through the same change but finally a violet or black pigment was deposited. The use of hypochlorite was discontinued in favour of peroxide plus small amounts of iron catalyst (Fenton's

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Table 1. *Change of concentration of sulphanilamide produced by oxidation with 6.5 ml. O₂ per 100 ml.*

Time in days	Ca(OCl) ₂							
	H ₂ O ₂ 17° light		17° light		17° dark		38° dark	
0	20.0	30.0	20.0	30.0	20.0	30.0	20.0	30.0
1	6.5	17.8	2.6	3.3	4.7	7.4	8.4	12.8
2	5.3	7.2	2.0	2.1	3.2	5.2	6.3	4.6
4	3.3	4.5	1.9	2.0	2.4	3.2	5.2	2.3
5	2.1	3.3	2.0	2.1	2.0	3.3	3.8	1.8
6	2.2	3.3	1.9	1.9	1.8	2.5	2.2	1.9

reagent), which more closely resembles the oxidizing system of the body. The effects of this combination on both sulphanilamide and sulphapyridine at room temperature with exposure to light are summarized in Table 2.

Table 2. *The catalytic effect of iron*

Time hr.	Sulphanilamide, mg. per 100 ml.				Sulphapyridine, mg. per 100 ml.			
	H ₂ O ₂		H ₂ O ₂ + 5 mg. FeSO ₄		H ₂ O ₂		H ₂ O ₂ + 5 mg. FeSO ₄	
0	20.0	40.0	20.0	40.0	20.0	40.0	20.0	40.0
1	19.4	39.8	18.8	38.4	20.1	39.5	19.0	38.6
2	19.2	39.2	15.8	37.8	19.6	38.7	19.0	38.8
3	18.9	38.5	13.2	33.2	19.3	37.5	19.2	34.1
4	18.2	37.8	12.7	26.7	18.8	35.3	17.0	28.2
24	15.2	32.8	1.8	23.0	16.5	34.1	15.0	25.0
48	8.5	28.6	1.9	7.0	13.3	22.5	8.1	20.0
96	6.6	23.0	1.6	6.8	9.3	18.3	7.2	17.0
240	2.2	6.4	1.5	6.2	6.7	10.8	6.5	12.1

The use of iron speeds up the reaction but with the same concentrations the same end point is reached without iron. The more rapid disappearance of sulphanilamide noted in Table 1 may be due to the larger amount of daylight falling on the solution, as the experiments recorded in Table 2 were done later in the year than the preliminary investigations of Table 1.

The results in Table 1 were obtained by simple diazotization as it was not then realized that this also estimated hydroxylamine compounds and it is possible that the action of light might lead to the further decomposition of hydroxylaminobenzene sulphonamide to *p*-aminophenol and other compounds; it is unlikely that peroxide in the dark is entirely without action on amines.

The experiments of Table 1 were repeated on sulphanilamide, hydroxylamine being estimated by adding 1 ml. acetic anhydride to 5 ml. of the solution, shaking well and allowing to stand for 5 min., then adding *N*/2 HCl and diazotizing in the usual manner. The acetic anhydride converts the amino group, if present, into the inactive acetamino group and the nitrous acid changes the hydroxylamine group to the amino group which is then estimated. To estimate the sulphanilamide, the hydroxylamine value is deducted from the value obtained for the first estimation which includes both amine and hydroxylamine.

p-Aminophenol may be estimated by the indophenol reaction the pigment being evaluated by placing the coloured solution in one cell of a Spekker photoelectric absorptiometer and taking the calibrated drum reading as a measure of the colour present. A combination of Ilford screens Micro 1 and 2 was used as being complementary to the pigment colour.

Table 3. *Effect of light on the production of hydroxylamine compound*

Time days	17°				38°	
	Light		Dark		Dark	
	Sulphanil- amide	Hydroxyl- amine	Sulphanil- amide	Hydroxyl- amine	Sulphanil- amide	Hydroxyl- amine
0	20.0	0.0	20.0	0.0	20.0	0.0
1	18.1	0.5	19.6	0.4	18.7	0.8
2	16.0	0.6	18.9	0.7	16.2	3.6
3	2.8	4.0	16.0	4.0	15.6	4.0
4	1.8	3.7	15.8	3.6	14.4	5.1
7	2.0	4.0	14.0	3.6	12.7	6.2

Table 4. *Estimation of all products of oxidation at 17° in daylight*

All results are expressed as mg. per 100 ml.

Time hr.	Sulphanil- amide	Hydroxyl- amine	<i>p</i> - Amino- phenol	Pigment drum reading	Sulpha- pyridine	Hydroxyl- amine	<i>p</i> - Amino- phenol	Pigment drum reading
0	40.0	0.0	0.0	0.000	40.0	0.0	0.0	0.000
1	39.7	0.0	0.0	—	39.6	0.0	0.0	—
2	39.2	0.0	0.0	—	39.0	0.0	0.0	—
3	38.6	0.8	0.0	—	38.0	0.0	0.0	—
4	38.0	1.2	0.0	0.000	37.2	0.0	0.0	—
24	32.0	3.4	0.0	0.077	32.0	2.4	0.0	0.105
48	25.0	8.5	1.0	—	18.2	5.3	2.6	0.176
72	21.1	10.2	2.6	0.228	14.4	6.1	3.2	0.245
96	15.0	12.1	5.8	0.302	12.1	5.9	5.3	0.389
120	3.2	11.9	4.1	0.348	4.6	7.8	5.1	0.541

Table 3 gives the results obtained by estimating sulphanilamide and hydroxylamine after oxidation in the light and in the dark at 17° and 38°. Table 4 shows the results of oxidation of the drugs with peroxide in terms of hydroxylamine compound, *p*-aminophenol and pigment in each case.

It is seen from Table 4 that the sum of the amounts of the drugs and of their oxidation products does not equal the original amounts of the drugs; the discrepancy becomes increasingly larger with time and corresponds to an increased colour in the solution.

The pigment from these solutions, and others in which it was prepared by the action of calcium hypochlorite, was separated by evaporating the solutions to dryness, extracting with alcohol, again evaporating, extracting the residue with ether and evaporating the ethereal solution.

The pigments were examined according to Rota's scheme for the examination of dyestuffs; the sulphanilamide pigments fell into the indophenol class, the same one as that of the pigment already isolated from the urine of sulphanilamide-treated patients. The sulphapyridine pigments could not thus be classified although they were reduced in the same manner as the pigments from sulphanilamide.

The separated pigments were soluble in blood fat and were absorbed on to washed red blood cells, imparting to them a chocolate colour such as may be seen in the blood of some patients who exhibited cyanosis.

The oxygen capacities of corpuscles with and without absorbed pigment were measured. It will be seen from Table 5 that the presence of the pigment does not affect the oxygen capacity. This is in accordance with the findings of Marshall & Walzl [1937].

Table 5. *Effect of pigment on O₂ capacity of blood*

	Original blood ml.	Blood + pigment ml.
Sulphanilamide pigment	11.8	11.6
Sulphapyridine pigment	14.82	14.97

Examination of alcoholic solutions of pigments with a hand spectroscope showed absorption in the red and blue regions. Red cells on which pigment had been absorbed showed no extra bands in the red, but in order to separate the two oxyhaemoglobin bands greater dilution of the blood was necessary owing to the non-specific absorption.

DISCUSSION

The pigments obtained by oxidation of sulphanilamide and sulphapyridine can be absorbed on to red blood corpuscles and produce an effect resembling that of blood from some cyanosed patients. The chemical nature of the pigments is still obscure but they arise as a result of oxidation of the drugs, sulphapyridine producing more pigment and less intermediate products than sulphanilamide.

The fluctuations of hydroxylamine and *p*-aminophenol shown in Table 4 are probably due to further oxidation of these compounds to pigment which appears at the same time as, or a little later than, they do, and which in the case of sulphanilamide is an indophenol pigment.

Light is necessary for the *in vitro* oxidation of the hydroxylamine compound but *in vivo* this is achieved by other agencies.

SUMMARY

Pigments have been obtained by oxidation of sulphanilamide and sulphapyridine which are absorbed by the blood fat of the cell envelope and this alters the colour of the blood without affecting the oxygen capacity or the spectrum. These pigments are the probable cause of cyanosis when both methaemoglobin and sulphaemoglobin are absent.

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