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Explanation for the Cyanosis of Sulphanilamide Therapy.*

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The extraordinary cyanosis shown by patients during sulphanilamide therapy has received no explanation.

Marshall,[1] King,[2] and others have demonstrated that the respiratory function of the hemoglobin is unaltered. Methemoglobin and sulphemoglobin have been found in some cases but in amounts far too small to explain the intense cyanosis. The plasma of cyanotic patients appears normal. Whole blood, whether arterial or venous, has a chocolate brownish tinge. Though such blood when aerated gains oxygen in normal amounts it does not become bright red but remains dark. This led us to seek for a colored derivative of sulphanilamide in the erythrocytes.

After various unsuccessful attempts we were led by the known helio-sensitivity of sulphanilamide-treated patients to try the effect of ultraviolet light. This enabled us to reproduce the phenomenon *in vitro* and to offer an explanation for the cyanosis.

We found that dilute solutions of sulphanilamide on very brief exposure to ultraviolet light develop a strong violet color. The nature of the chemical change is so far unknown.

When red cells are added to such solutions they at once adsorb the violet substance from the supernatant fluid and assume the color shown by the red cells of patients under sulphanilamide therapy. Plasma added to the violet solutions causes the color to disappear.

The violet substance is only the first of several colors produced by irradiation of sulphanilamide. It is developed only by irradiation of short duration, one half to one or at most 3 minutes. When irradiation is continued for longer periods the color rapidly changes to yellow, then brownish.

When the violet solutions are allowed to stand a slow spontaneous change occurs. In an hour the violet fades markedly and a more permanent pinkish purple of less intensity appears.

The violet color is only produced by irradiation of dilute sulph-

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1 Marshall, E. K., Jr., and Walzl, E. M., *Bull. J. Hop. Hos.*, 1937, **61**, 140.

2 King, F. H., and Leslie, A., *J. A. M. A.*, in publication.

anilamide solutions—of a concentration in fact approximating that in the blood of treated patients—namely, one to 15 mg per 100 cc. When more concentrated solutions are used the yellow color dominates from the start (Table I). In numerous repetitions of these experiments it was found that the intensity of the colors varied with the depth of the fluid layer irradiated.

TABLE I.
5 cc of Each Solution in 9 cm Petri Dish Irradiated for 60 seconds with 110 V.D.C. Hanovia Lamp. Distance 40 cm.

Mg sulphanilamide per 100 cc	Color developed
0.8	very faint violet
1.6	faint violet
5.0	violet +++
8.0	" +++
10.0	" ++++
16.0	blue, brownish red
32.0	dirty yellow
48.0	yellow ++
64.0	" +++
80.0	" +++

When dilute suspensions (2%) of red blood cells in sulphanilamide saline solution are irradiated they undergo the color change but do not absorb the violet color from the supernatant solution as do freshly added red blood cells. Possibly they are already saturated with the color substance. They show slight hemolysis as do all irradiated red cells.

When quantitative determinations by Marshall's method[3] are made of the sulphanilamide in the violet solutions they are found to have lost 30 to 60% of their sulphanilamide (Table II). Repeated series of irradiations showed that the percent of sulphanilamide changed depended on several factors such as duration of irradiation and the depth of the fluid layer. As Marshall's method depends on diazotization it is probable that the color change involves the NH_2 group. Pointing in the same direction is the increased acidity of the solutions after radiation (electrometric pH determination). Beyond this we can as yet offer no chemical explanation. The possibility of oxidation was considered: sulphanilamide mixed with peroxide of hydrogen shows no change, but on irradiation develops a reddish yellow color. Irradiation of some chemically related simple compounds such as aniline and sulphanilic acid, develops no color.

Whether any of the colored derivatives of sulphanilamide produced in the body will by its superior bactericidal power explain the

[3] Marshall, E. K., Jr., *J. Biol. Chem.*, 1937, **122**, 263.

TABLE II.
Concentration of Sulphanilamide in Aliquot Portions, Before and After Production of Violet Substance by Irradiation.

Before radiation, mg per 100 cc	After radiation, mg per 100 cc	% decrease
6.3	**2.7**	**53**
6.3	**3.4**	**46**
7.0	3.0	57
7.0	3.0	57
9.0	4.5	50
9.8	**7.0**	**29**
11.0	**6.4**	**42**
12.2	6.1	50

chemotherapeutic effect (insufficiently accounted for by the very feeble bactericidal power of the drug itself) remains to be ascertained.

In offering this phenomenon as the explanation of the cyanosis during sulphanilamide administration we do not suggest that light is the causative factor *in vivo*. It would seem that a transformation so easily produced by light *in vitro* might well be brought about by some of the rapid and powerful metabolic changes constantly occurring in the tissues.

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Skin Absorption of Dihydroxyestrin in Humans.

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The present investigation was undertaken to determine whether estrogenic hormone can be absorbed through the human skin. Absorption was estimated on the basis of the application to the human female of the Allen-Doisy test. Papanicolaou and Shorr[1] have demonstrated that the human vaginal secretions after the menopause or castration exhibit certain striking cytological features, characterized chiefly by the presence of small, round or oval epithelial cells ("deep cells") with rather large, well-staining nuclei and associated with a varying number of leucocytes. These investigators have shown that following administration of adequate amounts of estrogenic hormone the leucocytes and "deep cells" disappear and are

[1] Papanicolaou, G. N., and Shorr, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 585.