It would appear, therefore, that while the sulfonamide drugs are able to prevent deaths from acute infection with lymphogranuloma venereum, their action is not always curative. Recovered mice may live healthy lives and raise families over periods of 3½ months, but at the end of this time show signs of chronic hydrocephalus and yield active, although slightly less virulent, strains of virus from the brain and other tissues. The importance of this in human chemotherapy cannot be over-emphasized. The idea that patients, receiving such therapy and becoming clinically "cured", are cured despite persistence of positive Frei and complement fixation tests requires reconsideration. Studies are now under way on the relative sterilizing powers of the different drugs, on the duration of the carrier state, and on the possible transmission of the infection from infected mother to the foetus *in utero*.

13308

Sulfonamide Inhibition of Bacterial Luminescence.*

FRANK H. JOHNSON AND K. MOORE.

From the Department of Biology, Princeton University.

Luminescence intensity is inhibited at once on addition of sulfanilamide either to broth cultures, or washed cell suspensions of luminous bacteria in phosphate buffered NaCl solutions containing glucose as oxidizable substrate. At the respective optimum temperatures of marine species, *Achromobacter Fischeri* (25 to 28°C), *Photobacterium phosphoreum* (15 to 17°C), and a fresh-water species, *Vibrio phosphorescens* (28 to 30°C), a concentration of 100 mg % sulfanilamide greatly reduces the intensity of luminescence, with little or no effect on the rate of respiration (Fig. 1). Except at high concentrations of the drug the inhibition of luminescence is largely or completely reversible (Table I) by centrifuging and resuspending the cells in a drug-free solution. In all these respects the effects of sulfanilamide resemble those of typical narcotics, such as the urethanes[1, 2] and barbital.[3] By analogy, it would be predicted that sulfanilamide will inhibit the luminescent

* Aided in part by a grant from the Penrose Fund of the American Philosophical Society.

1 Taylor, G. W., *J. Cell. Comp. Physiol.*, 1934, **1**, 297.

2 van Schouwenburg, K. L., *On Respiration and Light Emission in Luminous Bacteria*, Thesis, Delft, 1938.

3 Johnson, F. H., and Chambers, E. L., *J. Cell. Comp. Physiol.*, 1939, **13**, 263.

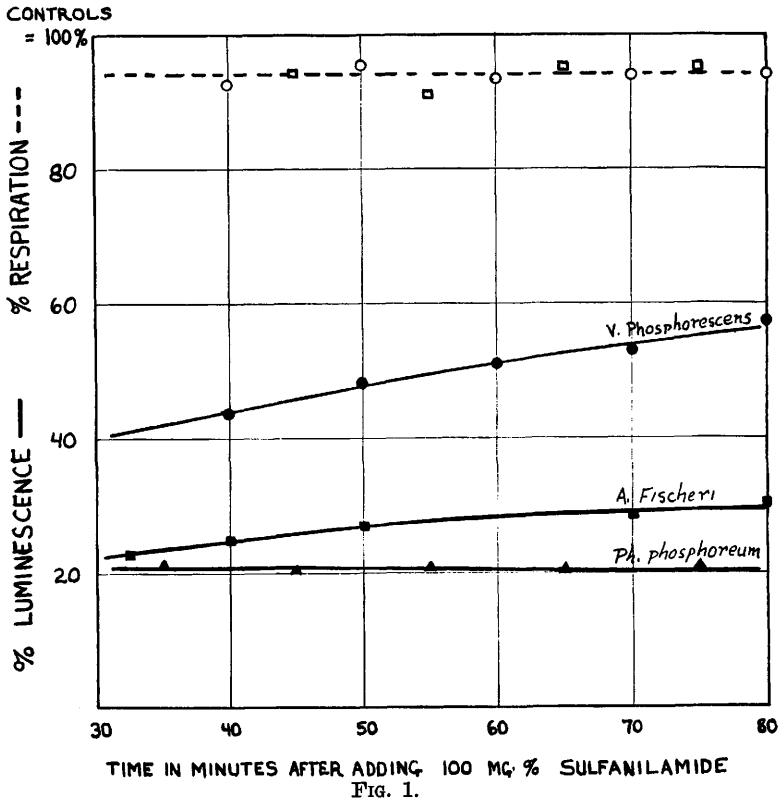

FIG. 1.

The effect of 100 mg % sulfanilamide on the luminescence intensity (solid points and lines) and rate of oxygen consumption (hollow points and broken lines) of three species of luminous bacteria at their respective optimum temperatures: *Photobacterium phosphoreum*, 15.5°C (triangles); *Achromobacter Fischeri*, 28°C (squares); and *Vibrio phosphorescens*, 28°C (circles). All suspensions were washed twice with NaCl solution of optimum tonicity (3% NaCl for the first two species, and 0.9% NaCl for the third) and resuspended in phosphate buffered NaCl solution, at a pH of 7.30, containing 0.01 M. glucose. The results are expressed as per cent with respect to a control without sulfanilamide, for both oxygen consumption (measured by Warburg respirometers) and for luminescence intensity (measured by a modified Leeds and Northrup MacBeth Illuminator).

oxidation of purified *Cypridina* luciferin and luciferase *in vitro*.[4]

Growth of luminous bacteria in nutrient broth is prevented by high concentrations of sulfanilamide (500 mg %), while lower concentrations retard growth and sometimes allow the development of cultures which do not luminesce. In sub-cultures of the latter, luminescence reappears, showing that no permanent change in the characteristics of the organism has taken place.

Concentrations of sulfanilamide which reduce luminescence intensity to as little as 10% cause neither an appreciable bactericidal

[4] Harvey, E. N., *Erg. d. Enz. Forsch.*, 1936, **4**, 365.

TABLE I.

Inhibition of Luminescence Intensity on Adding Sulfanilamide to Broth Cultures of *Photobacterium phosphoreum* at 25°C, and the Reversal of Inhibition by Centrifuging, Washing Once with NaCl, and Resuspending the Cells in a Medium Without Sulfanilamide.

		At start		After washing out the sulfanilamide	
Vessel	Conc. sulfanilamide in mg%	Luminescence intensity (arbitrary units)	% inhibition	Luminescence intensity	% inhibition
1	0 (control)	26.0	....	18.8	....
2	50	9.5	63.4	15.3	19.7
3	0	26.0	....	17.5	....
4	500	1.3	95.0	3.9	77.7

TABLE II.

Effect of 250 mg% Sulfanilamide on the Luminescence Intensity and Viability of Cells in a 24-hour Broth Culture at 15°C of *Ph. phosphoreum*. Temperature of Cultures During Experiment Was 23.5°C.

	Control			Plus sulfanilamide		
		Plate count 1:400,000 duplicate plates			Plate count 1:400,000 duplicate plates	
Minutes after adding sulfanilamide to culture	Luminescence intensity (arbitrary units)	Total colonies	Luminous colonies	Luminescence Intensity (same units)	Total colonies	Luminous colonies
5-10	11.7	113	90	1.6	110	92
		121	90		92	82
20-26	11.5	121	90	1.2	115	75
		108	86		105	62
35-45	9.1	123	79	<1.0	112	88
		105	89		120	94
50-60	8.2	121	89	<1.0	105	60
		119	98		84	77
70-75	7.7	115	86	<1.0	113	89
		143	115		110	96

effect, nor a reduction in number of luminous colonies in the plate count (Table II).

Para-aminobenzoic acid[5] at high concentrations (50 mg %) inhibits growth and luminescence, but exerts anti-sulfanilamide effects on broth cultures at much lower concentrations. Para-aminobenzoic acid does not, however, have any appreciable effect on the sulfanilamide inhibition of luminescence in mature cultures or washed cell suspensions, over a range of 0.001 to less than 0.0000001 M. It thus appears that PAB acts primarily through an effect on growth, and cannot reverse the narcotising action of sulfanilamide on the luminescence of normal cultures.

[5] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.