

Table 3. Radioactivity in liver polyamines in sham-operated and partially hepatectomized rats after injection of 2.5 μ C of 5- 14 C-DL-arginine. The animals used in this experiment weighed 110 to 120 g. Injection at 20 h after operation, analysis 4 h later.

Animal No.	Specific activity cpm/ μ mole			
	Sham-operated		Hepatectomized	
	Spermidine	Spermine	Spermidine	Spermine
1	100	40	1 050	100
2	370	50	1 020	360
3	280	50	1 080	360
4	320	100	1 110	250
Mean	270	60	1 065	270

increased during liver regeneration. Ornithine and putrescine or agmatine may be formed as intermediates.

It was stated previously that we have indirect evidence that spermidine acts as a precursor in spermine synthesis.^{1,2} The incorporation of 14 C-spermidine (now commercially available) into spermine was shown in regenerating rat liver. In a preliminary experiment after injection of 5 μ C of 14 C-spermidine at 22 h post-operatively, the radioactivity of liver total spermidine decreased from 14×10^5 cpm at one day after administration to 4.4×10^5 cpm at 5 days and that of liver total spermine simultaneously increased from 1.2×10^5 to 3.3×10^6 cpm. 5 days after injection the specific activity of spermine exceeded that of spermidine.

Studies on *in vitro* synthesis of polyamines as well as their fate in animal tissue are in progress in this laboratory.

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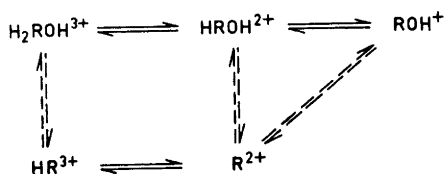
A Polarographic Study of Kinetics and Equilibria of Methyl Green in Aqueous Solutions

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The spectrophotometric investigation of the protolytic equilibria, hydration equilibria, and reaction rates of several basic triarylmethane dyes has been described in a series of papers emanating from this laboratory. Analysis of the dye solutions can also be made polarographically, and conventional polarography has been successfully used for a corresponding study of Methyl Green.

The reactions of Methyl Green in aqueous solutions can be summarized in the following reaction-equilibrium scheme (*cf.* Refs. 1, 2):



$\text{R} = [(\text{CH}_3)_2\text{NC}_6\text{H}_4]_2\text{C}-\text{C}_6\text{H}_4\text{N}(\text{CH}_3)_3$.

Whole arrows denote proton transfer reactions proceeding too rapidly to be measured; dashed arrows denote reactions with water or hydroxide ions proceeding at a measurable rate. The equilibria and the reaction rates are described by a set of equilibrium constants and rate constants, which are defined in Refs. 1, 2.

The literature about the polarographic behaviour of triarylmethane dyes is rather scanty and the published papers are mainly concerned with the electrode processes rather than the use of polarography for analysis.³⁻⁷ Methyl Green is reducible at the dropping mercury cathode, giving one wave within the pH-range 0-10. At $\text{pH} \geq 4$ the polarograms show tendencies to a separation into two waves. This separation seems, however, to be caused by the adsorption of the reduction product on the electrode surface. This conclusion is drawn from electro-capillary curves, which show a minimum roughly corresponding to the rise of the wave. The wave heights represent the concentrations ($[\text{R}^{2+}] + [\text{HR}^{3+}]$) and are directly proportional to the over-all dyestuff concentration (and accordingly ($[\text{R}^{2+}] + [\text{HR}^{3+}]$)) at concentrations greater than about 10^{-4} M. At lower concentrations there are deviations from the proportionality, which might be due to the graphical evaluation of the polarograms. The deviations are, however, too great to be accounted for only by this fact. They also might be due to the formation of polarographic maxima, which were not eliminated.

The constants were determined at the over-all dyestuff concentration $C = 75 \times 10^{-6}$ M. This value was chosen as a compromise between the maximum solubility of Methyl Green Perchlorate and a reasonable precision of the measurements. The temperature was 20.0°C, and the ionic strength was 0.50 M. Potassium chloride was used as a base electrolyte and to keep the ionic strength constant. The polarograms were evaluated graphically.

A detailed description of the experimental procedure, the calculation of the constants, and the polarographic behaviour of Methyl Green will be published later. In this paper only the obtained values of the constants are given, compared with the values obtained spectrophotometrically by Cigén (*cf.* Ref. 1, 2) at the

Table 1. A comparison between spectrophotometrically and polarographically determined values of the equilibrium constants and rate constants of Methyl Green in aqueous solutions. $T = 20.0^\circ\text{C}$. Ionic strength = 0.50 M.

	Polaro- graphically $C = 75 \times 10^{-6}\text{M}$	Spectrophoto- metrically $C = 1 \times 10^{-6}\text{M}$
K_1, M^{-1}	16.5	16.7
K_2	133	132
$K_3 \times 10^5 \text{ M}$	4.3	5.2
$K_4 \times 10^6 \text{ M}$	6.3	6.5
K_5	9.1	9.3
k_1, min^{-1}	0.122	0.123
k_2, min^{-1}	0.00092	0.00094
k_3, min^{-1}	0.0063	0.0062
k_4, min^{-1}	0.058	0.058
$k_5, \text{min}^{-1} \text{M}^{-1}$	—	87
k_6, min^{-1}	—	1.5×10^{-6}

dyestuff concentration 1×10^{-6} M (Table 1).

The polarographic method can also be applied to the study of other dyestuffs of this type. The studies of Malachite Green, which are now proceeding, are, however, complicated by maximum formation and by the low solubility of the carbinol.

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