Effect of Methylene Blue on Blood pH, Oxygen and Carbon Dioxide Content* (32951)

THOMAS P. STOSSEL (Introduced by Robert B. Jennings)
Department of Pathology, Northwestern University Medical School, Chicago, Illinois 60611

Slow intravenous infusion of large doses of methylene blue to anesthetized dogs produced a prompt increase in peripheral hematocrit and in respiratory rate (1). Both splenectomy and sympathetic blockade prevented increases in hematocrit produced by intraperitoneal injection of methylene blue in rats, indicating that the spleen was the source of the increase in circulating erythrocytes (1). It was suggested that these doses of methylene blue might produce hypoxemia. The acute changes in pH and oxygen and carbon dioxide contents of the blood of rats after single intraperitoneal injections of methylene blue are described in this paper. The results indicate that hypoxemia, acidosis, and hypercapnia occur after injection of methylene blue.

Materials and Methods. Measurement of pH and hematocrit of capillary blood. Fourteen female Sprague-Dawley rats weighing 200–300 gm received a single intraperitoneal injection of 65 mg of methylene blue (Merck, USP) per kg of body weight administered in warm isotonic saline at a concentration of 10 mg/ml. This is the approximate 24 hour LD_{50} dose of drug. Blood samples were drawn from the tips of the animals' tails immediately before and 1 hour after the injection. Six rats which received 2 ml of isotonic saline by intraperitoneal injection served as controls. Hematocrit values were determined in triplicate on all samples by a micromethod (3). For the estimation of pH, duplicate samples were collected in heparinized capillary tubes and immediately aspirated into the chamber of a capillary microelectrode pH meter (Radiometer, Copenhagen).

Simultaneous measurement of central venous pH, oxygen and carbon dioxide content. Eleven male rats were injected with 65 mg/kg of body weight of methylene blue as described above. One hour after injection the animals were lightly anesthetized with ether. A midline abdominal incision was made and 2 ml of blood were drawn from the inferior vena cava via a 1.5-inch 21-gauge needle attached to a polystyrene syringe. The apparatus contained heparin from the end of the needle to the plunger, and no air was introduced with the blood. The blood samples were immediately analyzed for pH, oxygen and carbon dioxide content in a pH and blood gas analyzing system (model 113, Instrument Laboratories, Boston, Mass.). Nine male control rats received equivalent saline injections and blood samples were obtained and analyzed as above.

Results. Effect of methylene blue on capillary blood pH. Methylene blue lowered the blood pH of 13 of the 14 rats tested. The mean pH value for blood drawn from the tails of the rats before methylene blue injection was 7.34 $\pm$ SD 0.03. After injection of methylene blue the mean pH value was 7.24 $\pm$ SD 0.05. The mean difference of 0.105 is significant ($p < .001$). Saline injection had no effect on the pH of the blood.

Effect of methylene blue on central venous pH and oxygen and carbon dioxide content. The mean pH value for the venous blood of the control rats injected with saline was 7.41 $\pm$ SD 0.01, whereas rats treated with methylene blue had a mean pH value of 7.26 $\pm$ SD 0.03. The difference in pH is significant ($p < .001$). The mean oxygen content for the controls was 53.8 $\pm$ SD 10.5 mm Hg, and the mean oxygen content was 43.8 $\pm$ SD 4.2 mm Hg for the treated group. This difference also is significant ($p < .05$). The mean carbon dioxide content for the control group was 43.8 $\pm$ SD 1.2 mm Hg, and the mean value for the animals treated with methylene blue was 58.3 $\pm$ 4.9 mm Hg. The difference in mean carbon dioxide content is significant ($p < .005$).

Discussion. In the experiments described,

* This work was supported in part by grants from the National Institutes of Health HE 08729, 5-T1-GM-131 and the Chicago Heart Association.

intraperitoneal injections of methylene blue resulted in the simultaneous lowering of pH and oxygen content and an increase in carbon dioxide content of the venous blood of rats. Because decreased blood pH and oxygen and increased blood carbon dioxide are factors which stimulate an increase in ventilation (3), the blood changes measured in rats after methylene blue administration are consistent with the previous observations that intravenous methylene blue increases the respiratory rate of dogs (1,8). The decrease in blood oxygen supports the contention that methylene blue causes reflex contraction of the spleen secondary to hypoxemia.

Low blood oxygen in parallel with low pH and high carbon dioxide can result from central respiratory depression, impaired pulmonary gas exchange or blood flow, or from hypermetabolism. The increase in respiratory rate and minute volume observed in dogs following injection of methylene blue does not support the mechanism of central depression (1,8). It has been reported that methylene blue alone causes pulmonary edema (5); but other studies have shown that this drug prevents pulmonary edema (6). In the study described in this paper, no differences in histology of the lungs were noted between treated and control rats. The increased hematocrit following methylene blue administration (1) could increase blood viscosity sufficiently to impair pulmonary blood flow, but no data is available on this point. Measurements of arterial blood gas concentrations would be necessary to rule out impairment of pulmonary gas exchange.

The pH and blood gas values observed in this experiment also could be explained by hypermetabolism. Methylene blue has been shown to increase total body oxygen consumption (7) and carbon dioxide elimination (8) in the dog. Increased utilization of oxygen and increased production of carbon dioxide and acid metabolites could explain the alterations noted.

These changes were not caused by formation of methemoglobin. The doses of methylene blue used in this experiment neither produce methemoglobin nor lower blood oxygen-carrying capacity *in vivo* (2).

Summary. Injections of a single large dose of methylene blue intraperitoneally in rats significantly lowered the pH and oxygen and increased the carbon dioxide of venous blood. These effects were believed to be due to hypermetabolism.

I am indebted to the Department of Pharmacology, Baxter Laboratories, Morton Grove, Illinois, for the use of its pH and blood gas analyzer, and to the assistance and suggestions of Drs. P. B. Herdson, R. B. Jennings, and H. M. Sommers.

1. Stossel, T. P., Proc. Soc. Exptl. Biol. Med. **128**, 93 (1968).

2. Stossel, T. P. and Jennings, R. B., Am. J. Clin. Pathol. **45**, 600 (1966).

3. Natelson, S., "Microtechniques of Clinical Chemistry," p. 73. Thomas, Springfield, Illinois, 1961.

4. Gray, S. J., Science **103**, 739 (1946).

5. Lagrange, E. and Promel, R., Arch. Intern. Pharmacodyn. **79**, 445 (1949).

6. Cottsegen, G., Szam, I., and Csornay, M., Arch. Exptl. Pathol. Pharmakol. **234**, 126 (1958).

7. Skoda, A., Rowe, G. C., and Crumpton, C. S., Am. J. Physiol. **207**, 1279 (1964).

8. Heymans, J. F. and Heymans, C., Arch. Intern. Pharmacodyn **26**, 443 (1922).

Received Oct. 26, 1967. P.S.E.B.M., 1968, Vol. 128.