secreted onto the alveolar space prior to birth, and is further discharged after the onset of respiration.

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Alterations in Hematocrit and Respiratory Rate Induced by Methylene Blue* (32950)

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During an investigation of the effects of methylene blue on the formation of methemoglobin *in vivo* (1), striking and unexpected increases in venous and arterial hematocrit were observed in both dogs and rats. In the present study, hematocrits, respiratory rate, blood pressure, and heart rate were studied in dogs given intravenous infusions of methylene blue. The increases in hematocrit observed previously were confirmed, and striking increases in respiratory rate were observed.

In order to determine whether splenic contraction could account for the observed increase in hematocrit under such conditions, methylene blue was administered to normal and splenectomized rats. Splenectomy abolished the increase in hematocrit indicating that contraction of the spleen is the probable source of the change in erythrocyte concentration. As splenic contraction is believed to result from sympathetic stimulation (2), the effects of drug-induced sympathetic alpha block on the response to methylene blue also were analyzed.

Materials and Methods. Dogs. Three female dogs weighing from 12 to 17 kg were anesthetized with 30 mg of sodium pentobarbital (Nembutal) per kg of body weight. Over a period of exactly 25 min, 50 ml of freshly prepared warm (32–37°C) methylene blue (Merck, USP) solution in isotonic saline was infused by a dual infusion pump (Harvard Apparatus Co., Inc., Dover, Mass.) via a polyethylene catheter inserted into a foreleg vein. In each case the dose was adjusted to 20 mg of

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TABLE I. Effect of 25 Min Methylene Blue and Saline Infusions on Venous Hematocrit and Respiratory Rate of Dogs.

Treatment	No. of dogs	Hematocrit before MB (%)	Hematocrit after MB (%)	Respiratory rate before MB	Respiratory rate after MB
MB infusion	3	43 ± 1[a]	49 ± 2	12 ± 2	30 ± 8
Saline infusion	3	44 ± 3	43 ± 3	12 ± 2	10 ± 4

[a] Mean ± SD.

methylene blue per kg of body weight. Blood samples, each of 3 ml, were taken from a polyethylene catheter in the inferior vena cava or from the femoral artery immediately before the infusion, and then every 5 min after the beginning of the infusion up to 30 min, and again at 45 and 60 min. Microhematocrit determinations (3) were performed in triplicate on each of the samples. One month later, the experiment was repeated employing the same dogs, but substituting isotonic saline for methylene blue as the infusion material. During each infusion the heart and respiratory rates and venous and arterial blood pressures were recorded on a direct-writing polygraph (Grass Instrument Co., Quincy, Mass.).

Rats. Forty-one Sprague-Dawley rats, each weighing from 100 to 200 gm, were divided into two groups. In the first group of 22 females, splenectomies were performed on 10, and the other 12 served as controls. Four days after splenectomy, each rat received an intraperitoneal injection of 65 mg of methylene blue per kg of body weight administered in isotonic saline at a concentration of 10 mg /ml. This is the 24 hour approximate LD_{50} dose of drug (1). Blood samples were taken from the tips of the tails immediately before and 60 min after injection for microhematocrit determinations which were performed in triplicate. In the second group of 19 males, 15 mg/kg of body weight of phenoxybenzamine (Dibenzyline, Smith, Kline and French) was administered in 1% gum tragacanth by gavage to 11 rats. At this dose epinephrine exerted no pressor effect. Three hours later, methylene blue was injected intraperitoneally as described above. The other 8 rats of this group received methylene blue only. Microhematocrit determinations were performed in triplicate on blood samples obtained immediately before and 1 hour after injection of methylene blue in each case.

Results. Dogs. Infusion of methylene blue produced a mean peak increase of 6% ± 2 in both arterial and venous hematocrit (Table I). This increase began 10–15 min after the beginning of the infusion, was miximal at 25–30 min, and remained elevated for about 3 hours. No changes in hematocrit were observed in control dogs during infusions of saline. The mean respiratory rate increased from an average of 12 to about 30/min approximately 5 min after starting infusion of methylene blue. This accelerated rate decreased slowly and returned to normal about 60 min after the beginning of the infusion (Table I). No marked changes in the ventilation rate occurred during the saline infusions. The heart rate and venous and arterial blood pressures varied considerably but erratically during and after the infusions of methylene blue and saline.

Rats (Table II). *Splenectomy.* In all unsplenectomized rats, a mean hematocrit increase of 7% was recorded after injection of methylene blue ($p < .001$). In splenectomized animals, the hematocrits increased slightly in

TABLE II. Effect of Splenectomy and Phenoxybenzamine on the Hematocrit Response of Rats to Intraperitoneal Methylene Blue.

Treatment	No. of rats	Hematocrit before MB (%)	Hematocrit after MB (%)
Splenectomy	10	46 ± 3.8[a]	48 ± 6.1
Normal	12	47 ± 3.4	56 ± 5.2
Phenoxybenzamine	11	41 ± 2.1	40 ± 2.3
Normal	8	43 ± 3.3	48 ± 5.1

[a] Mean ± SD.

six, decreased in three, and remained unchanged in one following injection of methylene blue. The mean change in hematocrit for all splenectomized rats receiving methylene blue, an increase of 2%, is not significant.

Phenoxybenzamine. A slight decrease in hematocrit occurred after injection of methylene blue in rats pretreated with phenoxybenzamine, while the hematocrits of the controls receiving no phenoxybenzamine demonstrated an average percentage increase of 12% after injection of methylene blue ($p < .005$).

Discussion. The results of these experiments confirmed that methylene blue increases the hematocrit when administered intravenously to dogs and intraperitoneally to rats. Methylene blue also was shown to accelerate the respiratory rate of dogs.

The effectiveness of splenectomy in preventing the hematocrit increase indicates that the spleen is the primary source of the hematocrit effect. Moreover, a 15.8% increase in red blood cell concentration reported as the result of splenic contraction in dogs subjected to complete obstruction of the trachea (4) is very similar to the 15% increase in hematocrit noted in dogs in this experiment. The fact that splenectomy lowers resistance to carbon monoxide poisoning in cats (5) may explain the reported efficacy of methylene blue as a therapeutic agent in carbon monoxide poisoning in rats (6).

Sympathetic blockade was more efficient than splenectomy in preventing increases in hematocrit, suggesting that contraction of the spleen after injection of methylene blue may be a secondary effect. The fact that tonic contraction of the spleen and other hemoconcentrating mechanisms are mobilized in conditions of lowered blood oxygen (4,7) suggests that hypoxemia may develop after injection of methylene blue *in vivo*.

Another possible mechanism by which methylene blue could cause splenic contraction is via potentiation of the action of endogenous amines. Methylene blue is an inhibitor of monamine oxidase and potentiates the pressor action of epinephrine, norepinephrine, and other amines (8). However, the failure of methylene blue infusion alone to increase blood pressure consistently renders this explanation unlikely.

Summary. The hematocrits of anesthetized dogs increased and their respiratory rates rose during intravenous infusion of 20 mg of methylene blue per kg of body weight. Blood pressure and heart rate responses were variable. In unanesthetized rats, intraperitoneal injections of 65 mg of methylene blue per kg of body weight induced marked increases in hematocrit. Splenectomy and phenoxybenzamine, an alpha sympathetic blocking agent, abolished the hematocrit response to methylene blue, indicating that reflex splenic contraction was the cause of the increase in hematocrit.

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