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Received July 15, 1963. P.S.E.B.M., 1963, v114.

Spectrophotometric Studies of Peritoneal Fluid in Strangulation Intestinal Obstruction.* (28636)

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Nemir and colleagues(1) demonstrated a hemin pigment in the loop fluid, peritoneal fluid, and blood of dogs just prior to death in experimental intestinal strangulation obstruction. There was no evidence that the spectrophotometrically observed pigment was the cause of death. It was suggested that the toxic agent appeared in the peritoneal fluid at approximately the same time as the changed pigment, and that the course of the toxic agent could be followed through that of the pigment. Subsequently, the pigment was observed in association with gangrenous intestine in but one of 18 clinical cases(2). Evaluation of the importance of the abnormal pigment in the pathogenesis of strangulation obstruction is difficult in view of the negative findings in clinical cases(3,4). A similar abnormal hemin pigment was observed in the plasma of dogs prior to death in irreversible hemorrhagic shock(3). Katz and colleagues (6), who were unable to demonstrate the abnormal pigment in experimental strangulation obstruction, concluded that any possible lethal action of strangulation fluid did not depend on the altered hemoglobin.

Both oxyhemoglobin and the abnormal pigment are characterized by a biphasic spectral absorption curve over the range 525 to 600 mμ. When a strong reducing agent (viz., sodium dithionite) is added, oxyhemoglobin is deoxygenated and yields a monophasic curve characteristic of reduced hemoglobin. With addition of dithionite to the abnormal pigment, a biphasic curve persists indicating an irreversible change in the hemoglobin. During a survey of strangulation loop fluids and peritoneal fluids, we observed a pigment, apparently similar to that initially reported, in the terminal peritoneal fluid of one dog(4). The presence of this pigment was not related to either bacterial flora or lethality of the peritoneal fluid. This pigment is of biochemical interest, and spectrophotometric studies demonstrating a relationship between the abnormal pigment and hemochromogen are described here.

Materials and Methods. Spectral absorption curves were obtained by reading optical density of appropriately diluted specimens in a Beckman DU spectrophotometer(4). Alkaline oxyhemoglobin was deoxygenated with sodium dithionite (sodium hydrosulfite, purified, J. T. Baker Chemical Co., Phillipsburg, N.J.). Total hemoglobin was determined by the cyanmethemoglobin method. Alkalinity was measured with a Beckman H-2 pH meter. The peritoneal fluid containing the abnormal pigment was aspirated from Dog #5430 which died 30 hours after a closed loop strangulation obstruction was created in a 30 cm segment of ileum(4). Dog #5432 was subjected to the same surgical procedure on the same morning and died 41 hours later.

* This investigation was supported in part by grants from the Edward G. Schlieder Educational Fund, and from Nat. Inst. of Allergy and Infect. Dis., U.S.P.H.S.

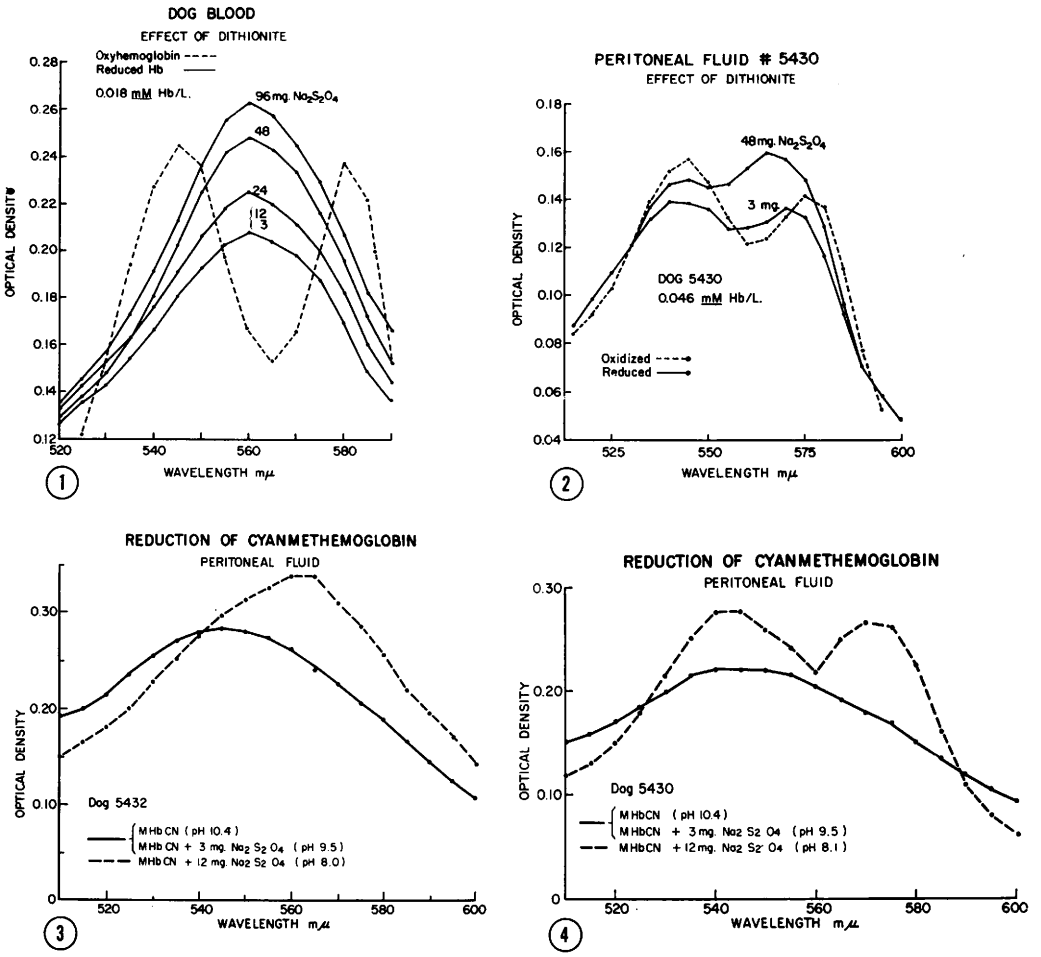

FIG. 1. A family of curves for reduced hemoglobin, showing a peak at 560 mμ which increased in optical density with increasing concentrations of reducing agent, demonstrates the effect of dithionite on an alkaline solution of dog blood.

FIG. 2. Effect of increasing concentrations of dithionite on biphasic reduced curves characteristic of the abnormal pigment.

FIG. 3. Cyanmethemoglobin derivative of a typical peritoneal fluid was produced by adding 10 ml of an aqueous solution containing 0.075% potassium ferricyanide and 0.212% potassium cyanide to 0.1 ml of peritoneal fluid.

FIG. 4. Cyanmethemoglobin derivative of peritoneal fluid containing the abnormal pigment was formed as in Fig. 3.

At autopsy each animal exhibited a well strangulated, but visibly perforated, loop. There was intense peritonitis with malodorous reddish-black peritoneal fluid.

Results and discussion. The spectral absorption curves for both oxyhemoglobin and the abnormal hemin pigment were biphasic over the range investigated. Oxyhemoglobin was deoxygenated by sodium dithionite, and gave a monophasic curve (Fig. 1). Peritoneal fluid from Dog #5430 was not deoxygenated by dithionite, and a biphasic curve persisted (Fig. 2). However, a change toward the spectrum of a reduced hemochromogen-like substance was observed after addition of the greater amount of dithionite. If the alkaline solution of peritoneal fluid were incubated at room temperature for 90 minutes before dithionite was added, the spectrum became even more hemochromogen-like. This solution was neutralized by increasing additions of dithionite.

The spectrum of the abnormal pigment has been observed to bear a superficial resemblance to that of either alkaline methemoglobin or oxidized globin-hemochromogen(1). These derivatives react with cyanide to yield the typical broad banded spectrum of cyanmethemoglobin. Accordingly, hemoglobin in the peritoneal fluids from Dogs 5430 and 5432 was converted to the cyanmethemoglobin derivative (Fig. 3, 4). Spectra were also recorded after reduction of the cyanmethemoglobin with dithionite. The curves for cyanmethemoglobin were broad peaked in the range 540 to 550 mμ. In the lower concentration of dithionite, curves for cyanmethemoglobin were unchanged. In the higher concentration of dithionite, the peak for the typical peritoneal fluid shifted to 560 mμ and was characteristic of reduced hemoglobin (Fig. 3). Normal dog blood responded similarly. Drabkin and Austin(7) have shown that, on reduction with dithionite, cyanmethemoglobin is converted to an unstable cyanide-hemoglobin which is rapidly split into hemoglobin and cyanide.

Although the cyanmethemoglobin derivative of the abnormal pigment appeared typical, the curve for the reduced cyanmethemoglobin derivative was biphasic and had a trough at 500 mμ (Fig. 4). Drabkin(8) has shown that upon reduction of the monocyanide derivatives of oxidized hemochromogens, the corresponding reduced hemochromogen is obtained. The reduced cyanmethemoglobin derivative in Fig. 4 is not a hemochromogen, but seems analogous to one. This then appears to be another property of the abnormal pigment which demonstrates a relationship to hemochromogens.

The inability of the abnormal pigment to be deoxygenated by dithionite has been cited as an extraordinary property for a derivative of hemoglobin occurring *in vivo*(1). The behavior of the reduced cyanmethemoglobin derivative is analogous to that of the monocyanide derivative of an oxidized hemochromogen, but is not identical. The evidence presented does not distinguish between absorption spectra obtained from a new hemoglobin derivative or a mixture of two or more known derivatives of hemoglobin, but seems to indicate that the abnormal pigment may be a partially denatured hemoglobin.

Summary. An abnormal pigment similar to the one initially described was found in a terminal peritoneal fluid from a dog with experimental strangulation obstruction. Peritoneal fluid from a dog similarly operated upon at the same time served as a control for subsequent studies. With increasing additions of dithionite, the spectrum of the abnormal pigment changed toward that of a reduced hemochromogen-like substance. Upon reduction, the cyanmethemoglobin derivative of the abnormal pigment reacted analogously, but not identically, to a hemochromogen.

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Received July 15, 1963. P.S.E.B.M., 1963, v114.