THE CAUSE OF DEATH IN STRANGULATION OBSTRUCTION: AN EXPERIMENTAL STUDY

II. LETHAL ACTION OF THE PERITONEAL FLUID*

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IN OUR FIRST COMMUNICATION[1] we showed that, late in the course of strangulation obstruction in dogs intensively treated for shock, dehydration, and electrolyte imbalance, the peritoneal fluid changed from a pink, odorless, coagulable fluid to a reddish-black or black, malodorous, non-coagulable fluid. In view of the rapid demise of the animal after the appearance of the black fluid in the peritoneal cavity and its rapid absorption into the blood stream, it seemed likely that some lethal factor was present in this fluid. This report is concerned with the injection of the peritoneal fluid, removed from the strangulated animals at various intervals, into normal animals by both intravenous and intraperitoneal routes.

METHOD

Normal, unanesthetized dogs were used as recipient animals. In no case was the circulating blood volume decreased[2] before injection. The total amounts of fluid were in all cases delivered intraperitoneally between two and eight hours. Intravenous injections were given either by rapid drip into a leg vein if the amount was large, or slowly by syringe injection if the amount was small. Following injection, the animals were carefully observed, and the temperatures were followed in a number of the dogs. Peritoneal fluids were kept in the ice box at all times between sample collection and administration and warmed to room temperature just before administration. The fluid was, in all cases, injected unchanged within a few hours after collection. In those animals which appeared to be moribund, blood was taken for culture and for spectrophotometric analysis. All animals, except No. 11, were posted immediately after death.

RESULTS AND DISCUSSION

The injection of peritoneal fluid or gut contents from animals having a strangulation obstruction into normal animals has been used by many investigators as a method of determining the toxicity of these substances. While

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the criteria for "toxicity" have varied,[3-5] the peritoneal fluid, almost uniformly, has been shown to have essentially no effect on the recipient,[2, 6-12] even when injected in amounts up to 200 cc.;[4] and the late gut contents have been shown almost invariably to cause death in such small amounts as several cc.s.[8, 9, 13, 14] It is of interest to note, however, that Foster and Hausler[10] injected 80 to 100 cc. of loop fluid, filtered through sterile gauze only, from strangulated dogs dying between 7 and 12 hours, intraperitoneally into recipient animals without causing death.

In this study the criterion for toxicity of the peritoneal fluid was the death of the animal. Although occasional vomiting and diarrhea followed the injection of very large amounts of pink fluid, if death did not ensue the injection was recorded as having "no effect." Obviously, the amount of fluid to be injected was, as in the past, purely arbitrary. However, we feel that much clarification of this point may be gained by (1) a comparison of the total amounts of pink fluid and red-black or black fluid injected, and (2) our spectrophotometric studies.

A review of Table I reveals the following: The pink, odorless, coagulable peritoneal fluid which was present up until several hours before death in the strangulated animals did not cause the death of the recipient in any of six animals injected either intraperitoneally or intravenously in amounts up to 40 to 50 cc. per Kg. of body weight. We have injected intravenously as much as 425 cc. of this fluid into an animal over a period of one hour without any effects except a mild transient vomiting. In many cases this pink peritoneal fluid was withdrawn as late as 24 to 28 hours after strangulation.

It is of interest that the bacterial flora of this pink fluid was practically identical with the reddish-black, or black, fluid qualitatively and death was not caused by injection of the bacteria.[8, 11]

The animals which received the reddish-black or black fluid behaved in a different manner. Four of the seven animals so injected died, two at six and seven hours after intraperitoneal injection, and the remaining two at five hours and 30 to 40 hours after intravenous injection. Another animal (No. 13) died five days after receiving red-black fluid intravenously. It was thought that the death of this animal was due, at least in part, to distemper, and it is therefore not considered as one of the animals dying from the injection. It is true that large amounts (35 cc. per kilo.) of the black fluid were given intraperitoneally, but even these amounts were considerably less than the pink fluid given either intraperitoneally or intravenously. Of the two dogs that died following intravenous administration, it will be noted that one died five hours after receiving only 24 cc., and the other died between 30 to 40 hours after receiving 100 cc.

The reactions of the animals which died were similar in all cases whether the fluid was given intravenously or intraperitoneally. Up until around two hours after injection the animals appeared normal. After this period, however, their condition rapidly deteriorated, vomiting became more frequent and severe, and the animals were usually moribund several hours before death.

TABLE I.—*Lethal Action of the Peritoneal Fluid*

Recipient Animal	Weight (kg.)	Peritoneal Fluid Injected: Source	Hrs. Between Strangulation and Withdrawal	Character of Fluid	Organisms Present	Amount—Total Cc.	Amount—Cc./Kg.	Method	Interval of hrs. Given	Comment
1	9.0	331	3½–24	Pink, odorless, coagulable	Hemo. clostridia	400	45	I.P.	8	No effect
2	2.0	232	12–28	Pink, odorless, coagulable	Hemo. clostridia B. coli Salmonella	350	50	I.P.	4	No effect
3	7.0	357	24 or less	Pink, odorless, coagulable	B. Coli Hemo. strep.	280	40	I.P.	2	No effect
4	7.0	347	24 or less	Pink, odorless, coagulable	Hemo. clostridia B. coli Non-hemo. strep. Non-hemo. clost. A. aerogenes	210	30	I.V.	2½	No effect
5	6.7	295	24 or less	Pink, odorless, coagulable	B. coli Hemo. strep. Non-hemo. strep.	300	45	I.V.	3	No effect
6	8.5	365	20–25	Pink, odorless, coagulable	Hemo. clostridia B. coli Hemo. strep. Non-hemo. strep. A. aerogenes	425	50	I.V.	1	No effect
7	9.0	331	28–32	Pink, odorless, coag.	Hemo. clostridia 100	360	40	I.P.	3	No effect
			36	Reddish-black	B. coli 100					
			42	Black, foul, non-coag.	Strep. vividans 160					
8	10.0	235	28–32	Black, foul, non-coagulable	Hemo. clostridia B. coli Hemo. strep. Non-hemo. strep. Non-hemo. clost.	350	35	I.P.	1¼	Died 6 hrs
9	10.0	357	35	Black, foul, non-coagulable	Hemo. clostridia B. coli Hemo. Strep. Non-hemo. strep.	350	35	I.P.	6	Died 7 hrs.
10	7.3	357	35	Black, foul, non-coagulable	Non-hemo. clost. A. aerogenes B. proteus	24	3	I.V.	At once	Died 5 hrs. T = 107[1]
11	5.5	347	29 30	Red-black, non-coag. Black, foul, non-coag.	Hemo. clostridia Hemo. strep. Non-hemo. strep. 35 Non-hemo. clost. 65 A. Aerogenes B. proteus	100	18	I.V.	2	Died 30–40 hrs. T = 104[6] at 4½ hrs.
12	8.0	365	28¼	Red-black, faint odor, non-coag.	Hemo. clostridia B. coli	155	19	I.V.	2	No effect
13	8.3	365	28	Red-black, faint odor, non-coag.	Hemo. strep. A. aerogenes	125	15	I.V.	1	Died 5 days —distemper T = 105[2] at 4½ hrs.

The animal which lived for 30 to 40 hours was listless and obviously sick during this entire period. No temperatures were taken in the animals receiving the intraperitoneal injections, but it will be noted that the temperature was 107^{2}° F. just before death in one of the animals receiving an intravenous injection, and 104^{6}° F. at approximately the same time after injection in the other. This is interesting in view of the high temperature elevation observed terminally in the strangulated animals.[1] Blood cultures drawn 4.5 to 6.5 hours after the injection into the recipient animals which died were negative on direct inoculation onto blood agar in three cases, and positive for *B. Coli* in the remaining case.

The necropsy findings in the two animals which died following intraperitoneal injection were identical and limited to the abdomen. The peritoneum was mildly injected in areas. The peritoneal cavities contained 150 cc. (No. 8) and 275 cc. (No. 9) respectively, of a red-brown fluid somewhat similar to that injected. In both cases there was an intense injection of the mesentery, especially marked as the mesentery approached the bowel. The duodenal mucosa, and the jejunal and ileal mucosa to less extent, showed submucosal hemorrhages. Aside from a moderate congestion of all the abdominal viscera, no other pathologic findings were noted. No gross abnormalities were noted at necropsy in the animals receiving the intravenous injection.

While our overall results with injections of the peritoneal fluid clearly indicated the great toxicity of the black peritoneal fluid, nonetheless, there appeared to be marked variations in the toxicity of this fluid from different animals. Variations in the character of the black or red-black fluid, as suggested clinically by the different responses, was objectively confirmed by our spectrophotometric studies, and the cause for this variation was conceivably explained by a review of the intake and output data for the strangulated animals. The peritoneal fluid from dog No. 357 was lethal when only 24 cc. was injected intravenously. This peritoneal fluid gave a spectrophotometric curve (Curve 3, Fig. 1) very similar to the one given by the bowel contents from this animal, and would be expected since approximately 500 cc. of intraluminal contents passed into the peritoneal cavity in the last few hours of survival. The fluid from dog No. 347 was lethal to the recipient at 30 to 40 hours after 100 cc. intravenously and the change toward the characteristic abnormal spectrum was less marked. As we were completely aspirating the strangulated lumen in this animal, less fluid passed out into the peritoneal cavity, and therefore, the dilution factor by the pink fluid was greater. Direct evidence of this was obtained from the values for original concentration of total pigments as cyan-methemoglobin. The peritoneal fluid of dog No. 365 (no death in recipients with 155 and 125 cc. respectively intravenously) showed the least marked spectrophotometric changes (Curve 4, Fig. 1), and here again, the dilution factor was of considerable magnitude.

The degree of toxicity, as expected, was greater in those animals in which a greater amount of the lumen contents diffused through the wall into the peritoneal cavity. This is borne out by the fact that the gut lumen was almost

devoid of fluid at post mortem in two animals having highly toxic fluids (No. 235 and 357, Curves 2, 3, Fig. 1), whereas almost 600 cc. of peritoneal fluid in each was present despite the fact that the peritoneal cavity was evacuated as completely as possible less than four hours before death. Animal No. 347 developed almost 300 cc. of black peritoneal fluid despite the fact that 200 cc. of gut contents was removed one hour prior to death, and the peritoneal fluid from this animal was also lethal.

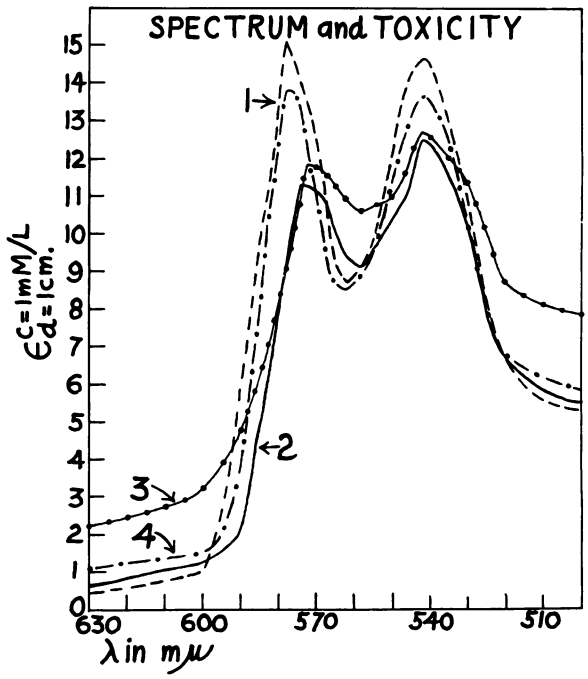

FIG. 1.—Data illustrating the relationship of toxicity to degree of spectral abnormality in comparison with normal oxyhemoglobin spectrum.

Curve 1. Absorption spectrum curve of unchanged oxyhemoglobin.

Curve 2. Absorption spectrum curve of peritoneal fluid (highly toxic) collected at 32 hours after strangulation from dog No. 235. Original concentration of total pigment as cyan-methemoglobin was 4.46 Gm. per 100 ml.

Curve 3. Absorption spectrum curve of peritoneal fluid (highly toxic) collected at 35 hours from dog No. 357. Original concentration of total pigment as cyan-methemoglobin was 1.14 Gm. per 100 ml.

Curve 4. Absorption spectrum curve of peritoneal fluid (relatively nontoxic), collected at 28 hours from dog No. 365. Original concentration of total pigment as cyan-methemoglobin was 3.24 Gm. per 100 ml.

We have stated[1] that the demonstration of an abnormal pigment, present first in the strangulated lumen, later in the peritoneal fluid, and finally in the blood, has been a useful method of following the course of events in the

strangulated animals. From the results herein reported, this finding assumes greater significance. It would appear that some relationship exists between the content of unidentified pigments responsible for the abnormal spectrum of the black peritoneal fluid and its toxicity. Some relationship is also indicated by the fact that one of the six animals which died following strangulation had only 40 cc. of black fluid in the peritoneal cavity at death and this fluid showed the most marked changes spectrophotometrically of any obtained. Also, it will be remembered that the altered curves were not obtained in the gut contents until around 12 hours. In view of the fact that Foster and Hausler[10] injected large amounts of less than 12 hour loop fluid into other animals intraperitoneally without causing death, a relation between the altered pigment and the toxicity of the fluid seems indicated.

CONCLUSIONS

1. The pink or strawberry colored peritoneal fluid, removed from animals with a strangulation obstruction, which owes its character to the presence of blood and unchanged hemoglobin, is non-toxic on injection into normal, recipient animals either by the intraperitoneal or intravenous routes even when rapidly administered in amounts up to 50 cc. per Kg. of body weight.

2. The late or black peritoneal fluid, which has been shown to be derived at least in part from the lumen of the strangulated gut is lethal on injection into normal, recipient animals by either the intraperitoneal or intravenous routes when administered in much smaller amounts than the pink fluid.

3. The toxicity of the peritoneal fluid samples removed in experimental strangulation obstruction bears a relationship of proportionality to its content of unidentified pigments responsible for the abnormal spectrum of the black peritoneal fluid.

4. It must be stressed that while the abnormal spectrum characterizes the toxic fluid, and, indeed, has served as a measure of the degree of toxicity of the fluid on injection into other animals, no evidence is at present at hand to identify the pigment itself as the toxic agent.

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