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The Red Blood Cell: An Essential Component of the Toxicity of Strangulation Intestinal Obstruction. (26906)

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It has been demonstrated by several investigators that the peritoneal exudate from dogs which have a strangulated loop of intestine is lethal when given to normal animals(1,2). Such fluid is not always fatal and we have attempted to find out why.

Our data from many dogs and rats revealed that 2 elements were constantly present in any strangulation fluid that was subsequently lethal to normal recipients: an *E. coli* level of at least 10^6 bacteria/ml and a hemogobin level of at least 3 g %. It did not appear that the presence of other bacteria regardless of concentration made any detectable difference. It did appear, however, that if the *E. coli* level fell below that stated, or if the hemoglobin level fell below 3 g % the fluid began to lose its lethal effect. The following experiments were designed to test this hypothesis.

Methods. If the above hypothesis is correct it should be possible to manufacture this lethal fluid in the laboratory. The following groups of experiments were done and the results checked by intraperitoneal injection of the fluid into the rat in a dose of 5 ml/kg. This is the same quantity of fluid used in our earlier experiments. All studies were carried out in male rats of the Sprague-Dawley strain and weighing 200-250 g.

I. The first group of 45 rats were given 5 ml/kg of a 24-hour broth culture of *E. coli*. The inoculum was adjusted to contain 1×10^8 organisms/ml.

II. The second group of 15 rats was given whole blood intraperitoneally 5 ml/kg.

III. The third group of 29 rats was given an intraperitoneal injection of *E. coli* plus whole blood. *E. coli* were grown in nutrient broth for 24 hours in presence of added whole blood so that hemoglobin concentration was at least 4 g %.

IV. The fourth group of 40 rats was given an I.P. injection of 10^8 *E. coli* grown in nutrient broth to which had been added rat plasma. The plasma was that amount present in the blood with a hemoglobin concentration of 4 g %. The amount of whole blood necessary to achieve this level was determined, then centrifuged and the resultant supernate added to the broth culture.

V. The fifth group of 174 rats received an I.P. injection of *E. coli* (10^8) in nutrient broth to which 4 g % of hemoglobin had been added in the form of washed red cells (red blood cells were separated by centrifugation and washed 3 times with saline solution).

VI. The final group of 10 rats were given an I.P. injection identical to group V except that *Clostridia welchii* replaced *E. coli*.

The number of bacteria injected was estimated in a Coleman Jr. spectrophotometer at 660 mμ. The actual number of viable bacteria injected was determined by subsequent dilution and plating of an aliquot of the injected culture. Quantitative counts were performed in a Quebec colony counter at 24 hours.

Hemoglobin level was determined using the cyanmethemoglobin technique read in a Klett colorimeter using a 420 filter.

Results. The results are summarized in Table I. When *E. coli* or the hemoglobin solutions were given independently of each other there was no lethal effect. If the 2

TABLE I. Results of Injection of Bacteria and Hemoglobin Concentrations into the Rat Peritoneal Cavity.

Group	No. rats	Bact. conc.	Media	No. killed	%
I	45	*E. coli* 10^8	Nutrient broth	0	0
II	23	None	*Idem* + Hgb 4 g %	0	0
III	29	*E. coli* 10^8	" + whole blood (Hgb 4 g %)	23	79
IV	40	*E. coli* 10^8	" + plasma	0	0
V	174	*E. coli* 10^8	" + washed RBC 4 g %	164	94
VI	10	Clostridia 10^7	" + washed RBC 4 g %	0	0

were incubated together the material became highly lethal. When plasma was used in place of hemoglobin the material was nonlethal (Group IV) and if bacteria other than *E. coli* were used, the material was nonlethal (Group VI). Subsequent experiments have shown that red cells from different species are equally effective in that the same highly toxic effects have been duplicated using washed dog red cells and washed human red cells.

Discussion. Our interest in development of a lethal fluid in the laboratory resulted from the fact that we were unable to produce a uniformly fatal fluid when short loop strangulation obstruction was produced in either the dog or the rat. If several fluids were pooled the resultant pool was usually fatal, but when individual fluids were used the recipient animals were killed in only 50% of instances.

A careful study was made of the quantitative bacteriology and chemistry of the fluid removed from the animals with a strangulating intestinal obstruction. The only two elements of all those measured which appeared to be consistent were the hemoglobin concentration and the level of *E. coli*. The experiments presented here confirm this hypothesis.

The evidence presented cannot be explained on the basis of data presently available. The possibilities are: a) the bacteria act on the red cell to release some factor which is lethal to the animal. b) The red cell in some way interferes with the animal's natural defense mechanism and permits the bacteria to be lethal. (In other words the red cell may act as an adjuvant similar to gum tragacanth(3) or mucin.) c) The bacteria and red cell interact to produce endotoxin. It is possible that the blood group substances which are muco polysaccharides are involved in this reaction. Studies are now under way utilizing various fractions of the red cell in an attempt to elucidate this mechanism.

Summary. The production in the laboratory of a fluid which simulates the "toxic" or lethal fluid of strangulating intestinal obstruction has been accomplished. This fluid requires a hemoglobin concentration of 4 g % and a level of *E. coli* of 10^6 organisms/ml or more. The fluid when injected into normal animals in a dose of 5 ml/kg intraperitoneally is almost uniformly fatal. The mechanism of death appears to be identical with that which follows intraperitoneal injection of raw strangulation fluid.

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