STUDIES ON THE ROLE OF BACTERIA IN IRREVERSIBLE HEMORRHAGIC SHOCK IN DOGS*

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USED LOOSELY, the term "irreversible shock" could apply to any condition in which the circulatory system can no longer be maintained at a level of functional efficiency sufficient to support life. In this sense, "irreversible shock" is almost as common as death. Against the background of modern shock research, however, the expression has gained a limited and fairly definite application to those cases of severe trauma or prolonged hemorrhage which cannot be made to survive by blood replacement up to and even well above normovolemia. Although such total irreversibility is rare in clinical practice, it is of considerable importance, because elucidation of this mechanism would add greatly to our fundamental knowledge of shock.

In laboratory shock, criteria of so-called "irreversibility" are difficult to define and largely empirical. Confusion has doubtless arisen in the past because of comparison of the results of research workers using totally different experimental parameters. For reasons already given,[7] we prefer a method in which the endpoint is determined by the animal's individual response, and indeed it has been essential for us to use this method for this particular investigation in order to achieve comparability with previous workers in the field.

The probable basic derangement in shock is a discrepancy in which the volume of circulating blood is substantially less than the capacity of the blood vascular system. Although Wiggers' data on central venous pressures have caused him to regard myocardial damage as of major importance in irreversibility to transfusion,[10] it is perhaps the majority view that such cardiac damage is an insignificant or terminal event, and that the important factors in shock are peripheral.[8, 11]

Much work has been done in an attempt to clarify these factors. Theoretically, the derangement could be caused either by loss of blood volume from the circulation, or by an increase in the capacity of the vascular bed from so-called peripheral vascular collapse. Although both these mechanisms may be involved, more attention has been paid of recent years to those causing peripheral vascular collapse, and Shorr and his co-workers have demonstrated a vasodepressor substance, elaborated chiefly in the anoxic liver, which they believe to be of significance in the problem of irreversibility.[9] In this connection it is interesting that Fine and his co-workers several years ago showed that dogs could be protected against irreversible shock by viviperfusion of the liver.[6]

However, Shorr's theory has not received universal support,[3] and more recent work by Fine and his group has produced some interesting results which they have interpreted as implicating a bacterial factor in the production of "irreversible" hemorrhagic shock in dogs.[2, 4] They base this thesis on the finding that the survival rate in their standard shock experiment is greatly improved by preliminary treatment of the dogs with large doses of certain antibiotics.

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The purpose of our investigation was, firstly, to attempt to confirm this work, and secondly, to try to establish the responsible bacterial factor, if any exists.

In our project, considerable difficulty was encountered at first in obtaining control data to match those of the original workers. For this reason only the last 50 experiments out of a total of about 200 are sufficiently comparable with Fine's series to report here. We are indebted to Dr. Fine for personal communications regarding his exact technic, which we have endeavored to follow closely.[1]

Healthy, afebrile mongrel dogs are used. In a few instances, owing to supply difficulties, the animals have been slightly below the 15 Kg. lower weight limit of the original workers.[2] The experiments are carried out in air-conditioned surroundings, with morphine premedication and local anesthesia, and using normal aseptic surgical technic. Manometers and reservoirs are autoclaved before use. The groin vessels are exposed on one side, and a plastic catheter is passed up the femoral vein and inferior vena cava to the level of the hepatic venous inflow. A branched cannula is inserted into the artery, with one limb connected to a reservoir and the other to a mercury manometer. A small dose of heparin is used to prevent clotting. The animal is bled rapidly into the reservoir which is adjusted at such a level that the blood pressure equilibrates at 30 mm. of mercury. Any tendency to deviate from this level is balanced by automatic bleeding or transfusion via the reservoir system. After a variable time, as the animal's compensation begins to fail, blood is progressively taken back from the reservoir. When 40 per cent of the maximum bleeding volume has returned spontaneously to the animal, the remainder is rapidly transfused and the experiment terminated. Almost invariably this transfusion produces a good pressor effect, but this is transient, and a very high proportion of the dogs die within a few hours.

Specimens for culture are taken of venous and caval blood before bleeding, immediately after transfusion, and from two to five hours after transfusion, depending on survival. If the animal is dead at the time of the final specimens, only caval blood is taken. The sampling blood loss per dog amounts to 12 to 18 ml. In our original protocol liver cultures were contemplated, but had to be abandoned owing to the high complication rate from needling the liver in these heparinized animals. Instead, we have recently carried out some liver studies in a group of five acute experiments.

The series presented here consists of 15 controls (which were spaced throughout the series), 15 dogs treated with oral aureomycin, 15 treated with intravenous aureomycin, and five acute experiments already mentioned. Antibiotic dosages recommended by Fine were employed[1, 2] as follows:

Oral Aureomycin, 5 Gm. daily by hand feeding for three days; 5 Gm. by stomach tube three hours before bleeding ("priming dose").

Intravenous Aureomycin, 500 mg. in 100 ml. normal saline by slow drip into the vena cava for one hour before bleeding.

BACTERIOLOGIC RESULTS

Blood Cultures. Cultures are made on pour plates, which are incubated aerobically, in reduced oxygen, and anaerobically. Where growth is obtained, a colony count is made to obtain rough quantitative results, and the organisms are then identified. Blood specimens are kept chilled between the time of taking and the time of culture, in order to inhibit bacterial growth which otherwise might occur at room temperature. Lack of this precaution led to a number of inaccurate results in our earlier experiments.

Cultural results are shown for the three groups in Tables I, II and III. An attempt has been made to indicate the relative amount of growth in each positive culture. The commonest organisms are *E. Coli, Alkaligenes fecalis, Aerobacter aerogenes,* and *Proteus vulgaris.* Occasionally other organisms appear, some of them doubtless contaminants. No *Clostridia* have been found.

In these experimental conditions, where the danger of contamination is difficult to control absolutely, one must avoid drawing too detailed conclusions from the bacteriologic findings. With this reservation in mind, the following comments are made.

The first venous specimen is sterile in every case. Most of the positive cultures are in the later specimens. Although it is tempting to regard this as an indication of bacterial proliferation in the hypoxic dog, it must be remembered that the chances of contamination increase with the duration of the experiment, and so the interpretation is doubtful. The caval specimens show more growth than their venous counterparts sufficiently often to suggest that this may indicate the passage of bacteria into the general circulation by way of the liver. The aureomycin-treated animals, as expected, show great reduction in the number of positive cultures. Two of the survivors show positive final blood cultures.

As indicated by Fine,[2] negative blood cultures are not necessarily reliable in this experiment, since the living plasma can destroy or immobilize bacteria with great speed. One might sum up these results by saying that they contribute comparatively little to the solution of the problem.

Liver Cultures. Liver cultures were taken from five dogs by means of needle corings obtained under anesthesia via an upper abdominal incision. They were cultured aerobically and anaerobically. Two weeks later the animals were subjected to the shock experiment, without antibiotics, and died or were sacrificed within one and one-half hours after transfusion. Similar liver cultures were then taken. The results are shown in Table IV.

By far the commonest organism was *E. Coli. Proteus vulgaris, Pseudomonas,* and occasional Gram positive cocci were also obtained. No Clostridia were found. This is a little surprising, as normal dogs are known frequently to harbour Clostridia in the liver. The explanation may lie in the fact that, in order to obtain more delicate results, only very small fragments of liver were used for these cultures.

TABLE I. *Results of Blood Cultures, Control Group.*

Dog #	Venous I	Caval I	Venous II	Caval II	Venous III	Caval III
1	0	0	+	+	–	–
2	0	++++	+++	+++	++++	++++
3	0	0	0	0	–	0
4*	0	0	0	0	0	0
5	0	0	0	0	–	0
6	0	0	0	0	0	–
7	0	0	0	0	+++	+++
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0	+	+	+	+
11*	0	0	0	0	+	+
12	0	0	0	+	–	+
13	0	+	0	++++	–	0
14	0	+	0	+	0	+
15	0	++++	++++	++++	++++	++++

*Survivor. +–Positive culture. 0–Negative culture.

TABLE II. *Result of Blood Cultures, Oral Aureomycin Group.*

Dog #	Venous I	Caval I	Venous II	Caval II	Venous III	Caval III
1	0	0	++++	++++	–	+
2*	0	0	0	0	0	0
3	0	0	0	0	–	0
4	0	0	0	0	–	0
5	0	0	0	0	–	0
6	0	0	0	0	–	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	–	0
10	0	0	0	0	0	0
11	0	0	0	0	–	0
12	0	0	0	0	0	0
13	0	0	0	0	–	0
14*	0	0	0	0	0	0
15	0	++++	0	++++	+++	++++

*Survivor. +–Positive culture. 0–Negative culture.

The findings suggest that some proliferation of organisms occurs in the hypoxic liver, although the possibility exists that there may have been persistence of infection following needling of the liver two weeks previously. It could be seen in most cases that the sites of the needling had not returned to normal at the time of the second culture.

TABLE III. *Results of Blood Cultures, Intravenous Aureomycin Group.*

Dog #	Venous I	Caval I	Venous II	Caval II	Venous III	Caval III
1	0	0	0	0	0	0
2	0	0	0	0	–	0
3*	0	0	0	0	–	0
4	0	0	0	0	–	0
5	0	0	0	0		0
6	0	0	0	0		0
7	0	·	0	0		0
8	0	·	0	0	·	··
9	0	0	0	0		0
10*	0	0	0	0	0	·
11	0	0	0	0	·	·
12	0	0	0	0	–	0
13	0	0	0	0	–	+
14	0	0	+ + +	0	–	0
15	0	0	+ +	0	0	0

*Survivor. +–Positive culture.
0–Negative culture.

Survival Results. Fine reports 88 per cent survival with oral aureomycin, 58 per cent with intravenous aureomycin, and 21 per cent in his controls.[2] Our figures for this series are shown in Table V.

It will be seen that there is no significant difference between the groups, although the slightly greater 36-hour survival in the oral aureomycin group probably indicates a slight trend in the same direction as Fine's results. Broadly, however, we have not succeeded in confirming the findings of the original investigators.

DISCUSSION

There are two possible interpretations of the fact that in the hands of the original workers the survival rate is improved by antibiotics. One is the hypothesis originally advanced by them, that this type of "irreversible" shock is due to the proliferation of bacteria in some locus, possibly the liver, which leads to toxemia and circulatory failure. The other possibility is that the course of the experiment may be influenced by some non-antibacterial side effect of the antibiotic, which for convenience has been called a pharmacologic action.

The first hypothesis seems to have little to support it other than the indirect evi-

TABLE IV.

Dog No.	Culture 1.	Culture 2.
1	0	+
2	0	+
3	0	+
4	+	+
5	+	+

0—Negative culture.
+—Positive culture.

TABLE V.

Group	Number of Dogs	Alive at 36 hours	Ultimate Survivors
Controls:	15	3 (20%)	2 (13.3%)
Oral Aureomycin:	15	6 (40%)	2 (13.3%)
Intravenous Aureomycin:	15	2 (13.3%)	2 (13.3%)

dence that certain antibiotics, when given in certain ways, have been found to improve the survival rate in this experiment. It does not account for a number of conflicting facts:

1. The antibiotic is not effective unless given in enormous amounts. The oral "priming" dose of aureomycin is equivalent to giving in one dose 17.5 Gm., or 70 capsules, to an adult human of average weight —far above the normal antibiotic range. Moreover, the antibiotic has to be given before the animal is bled, and is useless when shock is established. This makes the results of the experiment of doubtful validity even if protection is demonstrated.[5]

2. Neither in the original work nor in ours is there any convincing direct evidence that a bacterial factor is involved. Indeed, consideration of the effective versus the ineffective antibiotics makes it even more difficult to explain the phenomenon

on bacteriologic grounds. For instance, aureomycin is effective while chloramphenicol, with a very similar antibiotic spectrum, is not.[2]

3. Important evidence in favor of a pharmacologic factor would be afforded if protection against "irreversibility" were to be provided by aureomycin in which the labile antibacterial component has been destroyed. Owing to our inability to achieve results even with the active drug, we have not been able to undertake this experiment. Fine and his co-workers, however, have shown that such inactivated aureomycin has a protective effect which seems significant, even though it is less than that of the active drug.[2] It is difficult to see how this finding can be interpreted other than as showing that a pharmacologic side-effect of aureomycin is involved.

4. To us, the most potent argument against the bacterial theory of "irreversibility" is that we have been quite unable to reproduce the results of the original workers. The reason may lie in some unknown difference in technic or materials, for it is well known that minor variations in methods can add up to major variations in results. The fact remains, however, that our dogs died of so-called "irreversible shock," and that this outcome was practically uninfluenced by massive doses of aureomycin. We believe that if the broad concept were true, that this type of "irreversible" shock is due to bacterial factors and can be prevented by antibiotics, then that concept should hold good over a reasonable latitude of experimental conditions. The fact that we have failed to strike these conditions, in spite of conscientious effort, leads us to suspect that the phenomenon may be only a limited one, and not of fundamental applicability in the problem of "irreversible" hemorrhagic shock.

SUMMARY

1. Fifty dogs have been subjected to "irreversible hemorrhagic shock" by the technic of Fine, which is described. Fifteen animals were controls, 15 were treated with oral aureomycin, and 15 with intravenous aureomycin. Five were acute experiments for the purpose of liver culture. Blood cultures were taken throughout each experiment.

2. There was no difference in ultimate mortality between the three groups, and no evidence could be adduced for a bacterial factor in this type of "irreversible shock."

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