THE PRESENT POSITION AND PROGRESS IN RESEARCH IN THE PRODUCTION OF BLOOD FOR MASS CASUALTIES*

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SUMMARY: The present system of blood supply in the field depends on efficient communications because of the perishable nature and limited storage life of red cells.

In a mass casualty situation, when the supply of fresh blood cannot be maintained, the only alternatives are either procurement of blood from local donors or the use of stockpiles of blood stores in the frozen state. However, most of the available techniques for blood freezing are complicated and require time, considerable equipment and technical skill before it is ready for use.

Research being carried out by the Royal Army Medical Corps is aimed at developing a process whereby blood can be stored for long periods, using liquid nitrogen, but yet can be transfused directly after a simple thawing process without any further processing being required. The current state of development of such a process is outlined and some of the experimental, clinical and logistic aspects are discussed.

When given the opportunity to speak on this subject I carefully examined its implications; it became apparent that the key words were "Mass Casualties". I studied the opinions of many writers and these words are always linked with nuclear disaster; the picture which emerged was one of an isolated world in which communications did not exist and casualties were numbered not in thousands but tens of thousands as a result of the triple hazard of trauma, burns and radiation.

The medical services, who often seem immune to these hazards, having gathered the casualties together are concerned with sorting them into categories. Those in the first whose wounds are not too severe can be given only the simplest care by unskilled personnel. In the second they require urgent medical attention but have a good chance of survival. The final group are the gravely injured who will probably die; they must be set aside until those who will probably live have been attended to.

My task today is to examine the use of blood transfusion in this situation. A Field Surgical Pocket Book (1962) tells us that the aim of resuscitation is to prepare a casualty for surgery. Crosby (1964) states that blood transfusion used without surgery is generally useless. Thus a limiting factor which influences the situation is the number of surgical teams which are available to use blood. I am not in a position to suggest what that number might be. Redefined, the problem is one of provision of blood when forward distribution from base blood banks is inadequate for the surgical need. In the past the unit responsible for the supply of blood in the field was the Base Transfusion Unit (B.T.U.) Its primary function was to maintain supplies of blood to forward areas by establishing forward blood banks. These were supplied from the base blood bank which in turn received its supplies from outside the theatre, usually the United Kingdom. For most of the 1939-45 war the blood collection organisation was the Army Blood Supply Depot (A.B.S.D.), the present National Blood Transfusion Service springing from the original centre at Bristol. This system, which distributes such a perishable material, relies heavily on efficient communications by road and air.

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If blood could not be supplied by this method the only alternative until recently was the procurement of blood from local donors, often referred to as "keeping your blood on the hoof". This system which uses small bleeding teams to take blood from local donors is elegant in its simplicity, but depends on the ready availability of a source. In a nuclear disaster there are a number of possible sources, but it is difficult to estimate how useful they would be. Those who escape incapacitating injury will be expected to fight, and to bleed them would be psychologically unsound. Non-combatant troops would be working round the clock to restore some semblance of order, and to locate and bleed them in worthwhile numbers might be difficult. The lightly wounded have been suggested as a possible source. Indeed this category is likely to gravitate towards medical units in search of treatment. It seems quite reasonable to take a pint of blood from a man who cannot fight or work and who has lost only about this amount already. However, it would seem to demand sound clinical judgement to decide how lightly wounded a man is and how much blood he has lost, or more to the point, what he has left.

As a last resort the dead might be the most prolific of all the sources. Cadaver blood is traditionally associated with Russian transfusion practice (Vaughan, 1967), but its use is not as widespread as is often supposed. Blood coagulates after death but fibrinolysis occurs after one to two hours and the blood reliquifies. It may be removed from the body at this stage but, if removal is delayed, cell deterioration occurs. The risk of infection is high, particularly following open injury, so bacteriological control is standard practice. Thus a superficially attractive source of blood would be of little value in nuclear war.

Modest progress has been made in the provision of blood for mass casualties by mass blood grouping in time of peace. In a nuclear disaster the basic problem of blood group incompatibility remains and some attempt must be made to give blood of an appropriate group. Cross-matching is a refinement which will have no place, so the only alternative is pre-grouped soldiers. For this reason I will summarise the progress which has been made with the Army blood grouping scheme.

Blood grouping of all recruits was started on 1st January, 1964 at the A.B.S.D. and between 26,000 and 27,000 blood specimens have been grouped annually since that date. Originally 10 per cent of the subjects were serving soldiers, who had not been grouped previously and 90 per cent were recruits. In 1967 a new commitment was added, that of the Reserve Army. Although the number of regular soldiers and recruits grouped has grown less, the number of groups performed has stayed remarkably constant.

A soldier's blood group is embossed on his military identity card (MOD form 90), stamped on his identity disc and entered on his personal medical folder (F Med 4). The efficiency with which this is done is one of the most important parts of the whole procedure. Accurate documentation in the medical centre and repeated checks in the laboratory are of no avail if the last link in the chain is a Class III storeman with a hammer and letter punches hurrying to get to his N.A.A.F.I. break. It is the responsibility of the medical officer to enter the group on the F Med 4, but he is also responsible for ensuring that the personnel carrying out the other operations understand the implications of what they are doing.

A unit designed specifically for the collection of blood for mass casualties came into
being in 1967 and replaced the old Blood Transfusion Unit. This, 380 Blood Supply Unit
doing this and replaced the old Blood Transfusion Unit. This, 380 Blood Supply Unit
of the Reserve Army, can be broken down into six blood collecting sections, each of
which is further divisible into two collection teams. Thus twelve teams are available
which can be deployed over a large area under the direction of an administrative
headquarters, so that blood can be collected near the prospective recipients. Blood
would be collected directly into plastic packs labelled, at the time of collection, according
to the donors' identity discs. Groups would only be checked if time and circumstances
permitted. The speculation and planning which I have mentioned stem from the basic
fact that red cells under orthodox storage conditions have a life of only three weeks.
If blood could be stockpiled like other medical supplies the problem would not exist.
The extension of storage life has been one of the aims of blood transfusion workers for
many years and the R.A.M.C. in particular has maintained a great interest in this
field, which has culminated in the present research on blood freezing. When Sir Lionel
Whitby, who commanded the A.B.S.D. during the 1939-45 war, wrote the transfusion
history of that war, he began by enumerating the problems as they appeared in 1939.
The third on his list was "The initiation of work designed to prolong the period of safe
storage of blood". In 1939 the prolongation of storage life which was expected ranged
from a few days to a few weeks and only blood substitutes could be expected to store for
longer periods. Criteria for a blood substitute were suggested by two officers from the
A.B.S.D. who said, "Until blood can be stored for longer than a few weeks, blood banks
are faced with the choice of being either wasteful or inadequate. There is need therefore
for a blood substitute, suitable for the treatment of the war-wounded, which can be
stored for long periods, accumulated in large stocks and be immediately available
where casualties are likely to be received". (Buttle, Kekwick and Schweitzer, 1940).
I will pick out the essential phrases and apply them to a blood freezing process
suitable for field use.

The blood must be suitable for the treatment of the war wounded: it must be
 capable of being stored for long periods: it must be possible to accumulate it in large
stocks, and lastly, it must be immediately available where casualties are likely to be
received.

Assuming such a process could be developed, it would be a great advance in
providing blood for mass casualties. However, I should like to stress that there are no
grounds for supposing that frozen blood or local procurement from donors are exclusive
of each other. The two methods are likely to remain complementary and be valuable
sources of blood in a mass casualty situation.

The only effective way of slowing down the metabolism of red cells is to lower
their temperature. Blood will freeze at a temperature of minus 2°C to minus 3°C and the
ice crystals which form cause almost total destruction of the red cells by a combination
of mechanical damage and increasing salt concentration.

There are two possible solutions to this problem. The first is to add what are
literally large quantities of antifreeze to the cells so that the water is bound and is not
available for ice crystal formation. The second is to freeze the blood so quickly that
ice crystals have no time to develop and water passes directly into a vitreous state which
does not contain ice crystals of any appreciable size. Glycerol is one of the best agents
which has been discovered for binding water and if used in sufficient quantity will give
almost complete protection to red cells during freezing and thawing. Although this
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fact was discovered in this country nearly twenty years ago (Smith, 1950) the stumbling block which has prevented wide-spread adoption is the removal of the glycerol after thawing. A variety of methods of doing this have been developed but every one of them requires time, technical skill and complex equipment. The simplest of these techniques so far developed is that of Huggins (1964). This depends on the fact that in sugar solutions when no electrolytes are present, red cells will clump together and sediment extremely rapidly. Thus by washing the cells with sugar solutions sedimentation occurs and the supernatant fluid containing the glycerol can be decanted off after only a few minutes. Three washing cycles by this method remove practically all the glycerol and also the free haemoglobin, red cell ghosts, white cells, potassium and possibly the hepatitis virus. The equipment for the Huggins process is illustrated (Fig. 1).

After removal of the plasma the red cells are transferred to a special plastic bag which contains the cells during storage and the subsequent washing after thawing. As it will ultimately have to hold all the seven litres of wash solution required it has a capacity considerably in excess of this. A magnetic stirrer is incorporated in the bag which is used during the addition of the glycerol solution to the cells and also when the wash solutions are added after thawing.

The actual freezing operation takes place in a minus 80°C mechanical refrigerator and subsequent storage is in the same refrigerator. Processing after thawing is carried out in a special unit (Huggins cytoglomerator) which enables the supernatant wash solution to be decanted from the agglomerated cells by transference from one part of the plastic bag to the other, thus maintaining the operation in a closed system. A 40°C
water bath for thawing is incorporated in the equipment. The total processing time for each individual blood unit is approximately one hour, but the machine is designed to handle five units simultaneously.

To place this process in perspective, approximately 10 per cent of the blood used in the Massachusetts General Hospital is frozen and it is also being used in Vietnam. However, I should add that this is only at the base hospital at Da Nang and on the hospital ships Repose and Sanctuary where the use is mainly confined to cases where antibodies are a problem. I think that this is probably the best process which has been produced for peace-time use but it does not measure up to the criteria of ready availability where casualties are likely to be received. Time, technical skill, complex equipment and many gallons of wash solution are required to produce even a modest quantity of blood, so it has no place in a mass casualty situation. Unless the glycerol method can be made much simpler or possibly automated, the only hope of an acceptable long term preservation process suitable for war lies in rapid freezing and thawing. The basic discovery here is even older. Almost thirty years ago it was found that a film of blood on a glass coverslip could be frozen in microseconds, by dipping in liquid nitrogen, and the red cells recovered intact by thawing in warm saline (Luyet and Hartung, 1941). This is a phenomenon which is dependent on very thin films, and when volumes of the order of a pint are considered the heat transfer problems become insuperable.

The real advance came about ten years ago when it was discovered that much thicker blood films can be frozen when protective additives are employed (Strumia, Colwell, and Strumia, 1958). Since then the search has gone on for the perfect additive, which so far has eluded us.

The basis of liquid nitrogen freezing is that the blood is transferred to a flat aluminium container which is corrugated for rapid heat transfer and the blood is sealed in this container throughout freezing, storage and thawing. Freezing is accomplished in ninety seconds by shaking the container in a liquid nitrogen bath using a special blood processing unit (Fig. 2). The heat transfer rate during freezing is modified by an insulating film, applied to the exterior of the container. As the material used is water-soluble it is washed off automatically when the blood is thawed by shaking the container in a water bath at 45°C. Although this high temperature has to be used to maintain efficient thawing throughout the container, the blood only reaches 30°C because of the short period (ninety seconds) that is required for thawing. In practice the blood would be transferred to an empty plastic bag before use.

Many of the details of the liquid nitrogen process were studied by the R.A.M.C. using the additive polyvinyl pyrrolidone (PVP). This is a synthetic long chain polymer, used extensively by the Germans during the last war as a plasma expander. With this material blood can be frozen and stored indefinitely in liquid nitrogen, yet can be thawed and be immediately available for use without further washing of the cells.

This would seem to answer the criteria suggested earlier but there is still debate on the most essential criterion of all—the suitability for the treatment of the war wounded. This debate revolves around two features of the final product. These are that 40 g of PVP are used for each unit of blood and that 3 to 4 per cent of the cells are haemolysed by the freezing and thawing; their content of haemoglobin is liberated into the plasma, and is not removed before administration to the patient.

The first objection springs from a misconception about the molecular size of PVP.
This material can be produced with as large or small a molecular size as is desired. Many of the early preparations of PVP contained very large molecules which tended to be retained for long periods, if not indefinitely, in the reticulo-endothelial system. Modern preparations, however, contain smaller molecules which are completely excreted and this objection can be largely discounted.

The second factor, the presence of free haemoglobin, deserves more consideration. When free haemoglobin is introduced into a recipient’s circulation it combines with his haptoglobin to form a complex which will not pass the renal filter and eventual removal is by the liver. The haptoglobins have only a limited binding capacity, however, and as more haemoglobin is added the saturation point is reached and free uncombined molecules appear in the circulation; these will pass the renal filter and haemoglobinuria results. This point is reached after about two units of liquid nitrogen preserved blood have been given; the clinician becomes concerned because he associates this clinical sign with a transfusion reaction. This is not a transfusion reaction, but the physiological excretion of excess haemoglobin which the kidneys are quite capable of doing provided a reasonable urine flow is maintained. A comparable circumstance exists with sulphonamides and the contingency is recognised and allowed for.
A series of clinical cases investigated at The Queen Alexandra Military Hospital, Millbank, confirmed these facts but left unanswered the vital question—what happens when this blood is given to cases in established oligaemic shock? This type of case does not arise very often in this hospital and with one exception all the transfusions were to anaemic patients or to replace blood loss at operation. Further information on frozen blood in shock was sought in a series of animal experiments but before going on to give an account of these I must mention the one instance at The Queen Alexandra Military Hospital where frozen blood was used to treat the victim of a road traffic accident.

Mr. A was walking down the street one sunny morning when he stepped out between two cars directly into the path of a taxi. As he was on the hospital doorstep he was taken in and treated for his fractured ribs and humerus (L). As time passed his pulse began to rise and his blood pressure to fall and a diagnosis of ruptured spleen was made. A transfusion of four pints of blood was necessary before he was fit for operation and as he was O negative, two of these were frozen blood. At operation his ruptured spleen was removed—as was the ruptured kidney on that side, leaving the other one to excrete the free haemoglobin. This it did, without any trouble and Mr. A was discharged fit and well two weeks later. This was an isolated case, however, and for the next step we set out to produce shock in experimental animals and then to treat them with frozen blood.

Sheep were used for this work as they are easy to handle and a pint of blood can be obtained without distressing them. This was desirable as the freezing process is geared to pint quantities and a store of frozen blood from each animal was built up two to three months beforehand. Repeated acute haemorrhage so as to depress the blood pressure to 30 mm of mercury for one hour is lethal to these animals if no treatment is given. The experimental animals were treated with their own frozen blood, while a control group received fresh ACD blood. All the animals which were treated survived. The investigations which were carried out included continuous e.c.g. monitoring throughout shock and transfusion, while biochemical and haematological samples were taken at intervals. Eventually the animals were killed at periods varying from three to fourteen days and the renal histology was studied. There were no significant pathological differences between the animals treated with fresh ACD blood and those treated with frozen blood.

In spite of evidence that blood which is 3 to 4 per cent haemolysed could be used in an emergency when ACD blood was not available or was in short supply, there is an understandable reluctance on the part of clinicians to produce an iatrogenic haemoglobinuria. If dark red urine is to be avoided one is limited by the patient's haemoglobin binding capacity and blood frozen with PVP cannot be used in worthwhile quantities.

Current hopes for an improvement to the process are centred around a new material, hydroxyethyl starch (H.E.S.), which is being developed in the United States as a plasma expander. Preliminary reports are that 1 to 2 per cent haemolysis can be obtained with H.E.S. and samples are being obtained for evaluation at the A.B.S.D. Assuming H.E.S. did all that was claimed, how feasible is it to store and transport blood in such a futuristic refrigerant as liquid nitrogen?

In conclusion I should like to present a few facts on the logistic aspects of the problem.
Liquid nitrogen, far from being a laboratory curiosity, is today almost an industrial heavy chemical. It is produced by the thousands of gallons by industrial plants, but at the other end of the scale small portable equipment is available. Once produced, liquid nitrogen tends to revert to a gas by taking heat from the surroundings, but if heat is prevented from reaching it, it stays as a liquid. In practice it is stored in large vessels with vacuum insulated walls. However, as no insulation is perfect there is a small heat leak which results in slow evaporation of the liquid. Liquid nitrogen refrigerators are exactly the same except that only part of the interior holds liquid nitrogen while the remainder of the space is reserved for the product to be stored.

The liquid level is topped up at intervals and the whole system is independent of mechanical or electrical failure. Frozen blood could be stockpiled in large refrigerators coupled to tanks for bulk storage of liquid nitrogen, with automatic replenishment of the nitrogen in the refrigerator. Each individual refrigerator holding 200 units of blood (Fig. 3) has sufficient nitrogen in the base to give independent refrigeration for six weeks and thus detachment and transportation to other sites is quite feasible. Extensive road and air movement trials have already been carried out using a lightweight aluminium refrigerator with a capacity of 40 units of blood (Fig. 4).

I have tried in the time available to present a balanced picture of the present position and progress in the production of blood for mass casualties. Some of the details are new but the basic ideas of local procurement of blood and stockpiling are not. For my final words I should like to quote the words of two of this country’s pioneers.
Fig. 4. A portable liquid nitrogen refrigerator, capacity 40 units of blood. Total weight including blood and liquid nitrogen, 190 lbs.

in blood transfusion. Sir Lionel Whitby obviously visualised bleeding local donors when he wrote “There is a need to develop a stable grouping serum so that any unit or medical officer can be self supporting if need be.” Sir Alan Drury (1954) speaking of frozen blood, commented “Such stores of blood would be specially valuable for major emergencies in areas where blood donors are few in number or transport is likely to be disrupted”.

REFERENCES

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AXILLARY SEPSIS AND SUBCUTANEOUS STRING PHLEBITIS

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SUMMARY: Five cases of axillary string phlebitis are described.

All were treated by simple division of the taut string under local anaesthetic, with immediate relief of symptoms.

Axillary sepsis is a common condition which appears to occur more frequently in the humid tropical climate of Malaysia. This is possibly due to the reduced resistance of the skin to infection when it is kept constantly wet.

Complications of axillary sepsis are few, but one which is striking, because of the appearance of an unusual physical sign, is that of superficial phlebitis. The patient complains of limitation of movement of the axilla or elbow. Examination reveals the presence of a subcutaneous linear ridge crossing the axilla or antecubital fossa and having the appearance of a taut bowstring.

Mondor (1939) described superficial thrombo-phlebitis in the breast and pectoral region presenting as tender subcutaneous strings. Stevenson (1954) was the first to note a similar tight bowstring spanning the antecubital fossa which limited full extension of the elbow. The string was divided and proved to be a narrowed, sclerotic vein. Eastcott (1960) described eighteen cases of painful antecubital strings. Eight of these were in patients following radical mastectomy, whereas the others followed axillary sepsis. He showed histologically, and by angiography in one case, that the strings were veins. The condition resolved spontaneously in six weeks to six months.

Ashken and Cotton (1936), over three years, saw ten cases of subcutaneous string phlebitis of the pectoral, axillary and antecubital regions, the majority at the British Military Hospital, Taiping, Malaysia and associated with axillary sepsis. Biopsy material in five cases confirmed that the strings were veins which were the seat of a somewhat unusual inflammatory reaction, which led to a markedly thickened, fibrotic wall. They also allowed the condition to take its natural course, the strings remaining tender and painful for two to ten weeks.

Millar (1967) was the first to treat these allied conditions of Mondor's disease, axillary and antecubital string phlebitis by simple division of the tight strings. Complete relief of pain and disability was afforded by operation in five cases.

Case histories

Case 1. British male, aged 29 years. Right axillary boil for one week, treated by antibiotics and magnesium sulphate dressings. Two days previously he developed pain down the inside of the right arm and in the axilla on abduction. Examination showed a healing boil with moderate axillary adenitis. Abduction was painful at 45° and impossible above 90°. There was a tender subcutaneous string across the axilla, down the inside of the upper arm and across the medial side of the elbow. On abduction it stood out like a taut bowstring (Fig. 1). Under local anaesthesia the string was exposed above the elbow and was revealed as a tough, white fibrous cord about 1 mm in diameter. One cm of this was resected. At the moment of division the pain disappeared, and full
abduction was restored. The histological appearance of the resected specimen showed the string to be a thrombosed vein.

Case 2. Australian male, aged 18 years. Right axillary boil for four days, no treatment. Painful abduction for one day. Examination showed an axillary bowstring which was divided, using a tenotome, with complete relief.

Case 3. Maori male, aged 22 years. Repeated attacks of left axillary boils, treated both locally and by systemic antibiotics. Three-day history of painful left axilla but no obvious boil. Sent to hospital as a hysterical paralysis after he presented with almost total restriction of abduction. Examination revealed a taut bowstring of the axilla which was divided with a tenotome, resulting in complete relief of symptoms.

Case 4. Nepalese male, aged 40 years. Presented with restriction of movements of left arm for one week. Examination showed an axillary string and evidence of previous follicular sepsis. Divided using a fine scalpel with restitution of full movements.

Case 5. Nepalese male, aged 20 years. Painful right axilla for one week, several boils treated by magnesium sulphate dressings. Examination revealed that abduction was limited to 100° by a tender axillary string. This was divided with a fine scalpel with
considerable relief of pain but full movements did not return for a further three days, by which time the axillary sepsis had also settled.

Procedure

The operation is done under local anaesthesia. The subcutaneous string is put tightly on the stretch, and a fine tenotomy knife or No. 11 scalpel blade is inserted through a skin puncture wound close by. The blade is rotated until the cutting edge comes underneath the string, and is then advanced towards the skin, so that division of the taut string is done by sense of touch. The string is felt to give suddenly as it is divided, with dramatic relief of pain and restitution of full movements of the arm. The string itself remains tender to touch.

Discussion

Mondor's disease is a fairly well recognised condition of subcutaneous string phlebitis of the pectoral region. That a similar condition occurs on the body, in the axilla, and at the elbow is now quite clear. The presentation is the same, namely, tender strings felt just beneath the skin and causing limitation of movement of the arm or body. Biopsy results show that these strings are veins which are involved in a localised phlebitis leading to sclerosis of the wall and narrowing of the lumen (Stevenson, 1954, Eastcott, 1960, Ashken and Cotton, 1963). It is also clear that the affected vein must shrink longitudinally in order to produce its effects of limiting underlying movement. In many cases the string has developed following localised sepsis in the axilla, so this might well be an important aetiological factor.

Although the condition does resolve spontaneously over a period of weeks it is felt that simple division of the strings under local anaesthesia is such a quick and simple procedure, and affords such rapid and complete relief of pain and restriction of movement that it is the method of choice. It may be used by the doctor who firsts see these patients, and enables him to confer dramatic and gratifying relief by a simple procedure.

In no case has a string been reported on as due to lymphatic permeation by carcinoma, therefore division without biopsy is justifiable.

REFERENCES