(Fig. 1), and at necropsy his tricuspid valve was thought to be incompetent as well as stenosed. A similar case, but without proof at necropsy, is reported by Ferrer *et al.* (1953).

Association of Incompetence with Stenosis

The development of signs of tricuspid insufficiency in all patients with tricuspid stenosis who developed atrial fibrillation led us to suppose that the organically stenosed and obstructed valve is probably always incompetent to some degree. The findings of Ferrer *et al.* (1953), McCord *et al.* (1954), Whitaker (1955), and Hollman (1956, 1957), in general support this contention. However, Reale *et al.* (1956) doubt whether a marked systolic wave in the right atrial pulse necessarily indicates tricuspid incompetence, and they suggest that if the atrium is filled and stretched at the time of ventricular systole the regurgitation of even a very small amount of blood might produce a considerable systolic wave. Ten of their 13 cases came to operation, and "significant" regurgitation was detected in only one. A low cardiac output at the time of palpation of the valve, however, may obscure a regurgitant jet. Pantridge and Marshall (1957) reported incompetence in one of two cases of tricuspid stenosis at operation for tricuspid valvotomy.

However, the work of Müller and Shillingford (1954) and Korner and Shillingford (1957) suggests that a large positive systolic wave is in fact associated with a considerable volume of reflux estimated by the dye-dilution technique. Such a wave was regularly seen in our patients with atrial fibrillation, and at necropsy it seemed probable that the valves had been incompetent. The absence of this wave in sinus rhythm may have been partly due to better emptying of the atrium, which is then able to accept a regurgitant flow without conspicuous rise of pressure.

We believe, therefore, that incompetence is a very common accompaniment of stenosis, but is not necessarily invariable. This point has an important bearing on the surgical treatment of tricuspid stenosis, since valvotomy is unlikely to be wholly successful if appreciable incompetence is present, for it may be increased by valvotomy (Hollman, 1956), to the detriment of the patient. At the present time, tricuspid valvotomy is a well-recognized though uncommon operation (Brofman, 1953; Chesterman and Whitaker, 1954; Krook *et al.*, 1955; O'Neill *et al.*, 1954; Trace *et al.*, 1954; Bailey, 1955; Swan *et al.*, 1955; Gibson and Wood, 1955; Hollman, 1956; Reale *et al.*, 1956; Yu *et al.*, 1956; Pantridge and Marshall, 1957; Dalla Volta and Forattini, 1957). But, as Hollman (1956) points out, the risk of increasing incompetence is appreciable. We have no personal experience of tricuspid valvotomy, but we believe that the general applicability of the operation is small, and that the difficulties of selecting the suitable case are considerable. The operation should probably be reserved for patients whose symptoms are urgent and unrelieved by medical means.

Summary

Twenty-one proved cases of organic disease of the tricuspid valve have been presented. The diagnosis is discussed, with special reference to the modification in the clinical picture produced by other valve lesions. Clinical, haemodynamic, and pathological data are discussed. Special emphasis is laid upon enlargement of the right atrium, and a method of radiological measurement is presented. The importance of right atrial P waves in the absence of evidence of right ventricular hypertrophy on the cardiogram is stressed. Prolongation of the P-R interval is underlined as a valuable diagnostic pointer.

Reasons are given for concluding that tricuspid incompetence usually accompanies tricuspid stenosis, and the surgical implications of this are mentioned.

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EFFECT OF TEMPERATURE ON SURVIVAL OF BACTERIA IN BLOOD FOR TRANSFUSION

WITH A NOTE ON CONTAMINATION BY COLD-GROWING ORGANISMS

BY

J. D. JAMES, M.R.C.S., L.R.C.P.

Director, North London Blood Transfusion Service

AND

E. JOAN STOKES, M.B., M.R.C.P.

Bacteriologist, University College Hospital, London

From 1946 to 1949 the practice in the North London Blood Transfusion Centre was to transport blood collected at the donor sessions to the centre in vans without refrigeration. Thus, at the most outlying sessions, the first donor's blood was unrefrigerated for about eight hours before reaching the cold room at the centre; approximately 210,000 bottles of blood were so treated. In 1949 transport by refrigerator vehicles was considered.

When fresh blood is kept for four days at room temperature the red cells do not survive well on subsequent storage (Gibson *et al.*, 1947), but if it is allowed to cool slowly for a few hours before refrigeration it is unlikely to suffer, and, since the phagocytic and bacteriolytic processes in the blood are most active at body temperature, such treatment might even have definite advantages (James, 1954).

It was decided that a complete change to refrigerator transport should be delayed until evidence of its value could be obtained. In the meantime two refrigerator vans were employed in conjunction with five non-refrigerators. In fact, owing to the inconvenient size of the new vans when negotiating the narrow streets of London and some provincial towns, the approximate ratio in which they were used was 2:11.

During the period when both types of van were in use about 700,000 bottles of blood were collected and one death from infected blood was recorded, this bottle having been carried in a non-refrigerator van, but the

odds in favour of a non-refrigerated bottle causing such a mishap, irrespective of any effect of refrigeration, was of course 11 : 2. No other fatalities or severe reactions due to infected blood were reported, and in view of the very close liaison between the centre and regional pathologists, coupled with the absence of any reported incidents in the hospitals supplied, it is most unlikely that any occurred.

The object of the investigation reported here was to determine whether the use of refrigerator vans provides an additional safeguard or introduces an additional risk.

Routine Method of Collection

The skin over the site of venepuncture is cleaned with ether soap followed by 70% alcohol, which is allowed to dry, and then a small quantity of procaine is injected intradermally over the vein. A paper dust-cap, placed in position before autoclaving, is removed from the top of the bottle, which is then swabbed with 70% alcohol. A sterile airway needle and then the sterile needle of the taking set are pushed through the rubber wad at the sites exposed by two small holes in the metal cap. The sterile needle at the other end of the set is introduced into the vein through the anaesthetized area. A pressure of 70 mm. Hg is maintained on the upper arm by means of a sphygmomanometer.

After 420 ml. of blood has been collected into 120 ml. of glucose citrate (2% disodium monohydrogen citrate and 2.5% anhydrous dextrose) the needles are withdrawn. The top of the bottle is swabbed again with 70% alcohol and the puncture holes are sealed with a piece of sterile adhesive tape. The rubber wads are used only once. Finally a sterile plastic cap ("viskap") is placed over the bottle top. At one time these viskaps were carried in 10% "dettol," but the practice now is to autoclave them on reception at the centre and then to place them in jars containing 1 : 1,000 chlorhexidine ("hibitane") for use at the sessions.

All sterilization is done by steam pressure at 20 lb./sq. in. (1.4 kg./sq. cm.) for 30 minutes in autoclaves with automatic pressure and temperature recording.

The following bacteriological investigations were carried out.

1. Estimation of Number of Bottles Infected

During the last six years a proportion of bottles of blood collected by the centre, using the closed method described above, have been cultured either in the centre laboratory or in one of two other independent laboratories. Seven bottles of blood, one to seven days old, were cultured each month.

Five-millilitre samples of blood were delivered into each of two bottles containing 30 ml. of Hartley's nutrient broth plus 1% glucose and 0.5% potassium citrate. One was incubated at 37° C. and the other at room temperature (20–25° C.) for 48 hours. The cultures were then subcultured to blood agar which was incubated at the appropriate temperature for a further 48 hours.

Of 406 bottles tested, one was found to be infected. It had been carried in a refrigerator van in May, 1956, and yielded a micrococcus.

2. Preliminary Test of Bactericidal Power of Fresh Citrated Blood at Different Temperatures

Four tests were made with fresh blood from four donors and a peptone-water culture of the cold-growing pseudomonas (BP1) which had been isolated from the bottle of blood believed to have caused the fatal accident. The following two methods were used.

(a) Blood obtained by venepuncture was delivered in 2-ml. amounts into each of three bottles containing the appropriate volume of glucose citrate making 3 ml. in all. The same volume of peptone water in each of an equal number of bottles containing neutralized glucose citrate served as controls. All bottles were seeded immediately with a measured volume of a counted peptone-water culture of BP1. One of the bottles and its controls was then refrigerated at 4° C.; one was incubated at 37° C. for 12 hours before refrigeration, and the third was left at room temperature (18–23° C.) for 12 hours before refrigeration. All bottles were subcultured at intervals, and if after four weeks' refrigeration they remained sterile they were subcultured again after two days at room temperature as a further test of sterility.

(b) Citrated blood from a single donor was distributed in 3-ml. amounts in 180 bottles. Each of these and four peptone-water controls were seeded within 20 minutes of bleeding with approximately 10 viable organisms. Half the infected bottles and controls were then refrigerated; the other half remained at room temperature for 10 hours before refrigeration. There was no significant difference between white blood cell counts in the first and last samples distributed, so that it is unlikely that uneven distribution of white cells affected the results.

It seems clear from the results (Table I) that a proportion of samples of blood infected with this organism can sterilize themselves if kept for a time at 37° C. Evidence for steriliza-

TABLE I

	Inoculum per Bottle (3 ml.)	No. of Bottles Found Sterile		
		37° C. (12 hr.)	18–23° C. (12 hr.)	4° C.
Donor A ..	23 viable organisms	2/3	0/3	0/3
" B ..	10 " "	2/3	1/3	0/3
" C ..	10 " "	3/3	1/3	0/3
" D ..	9 " "	3/3	0/3	0/3
Peptone-water controls ..	9–23 " "	0/12	0/12	0/12
	Inoculum per Bottle (3 ml.)	No. of Bottles Found Sterile		
		18–23° C. (10 hr.)	4° C.	
Donor E ..	10 viable organisms	10/90	0/90	
Peptone-water controls ..	10 " "	0/2	0/2	

tion at room temperature is, however, slender, which is to be expected, since room temperatures are near the optimal for the growth of the pseudomonas but by no means optimal for the bactericidal activity of the blood. It was noticed that growth in blood which had been treated at room temperature could sometimes be observed about two days sooner than in the blood which had been refrigerated immediately. The inoculum in these small bottles was equivalent to 1,600–4,100 viable organisms per whole bottle (540 ml.) of blood.

3. Tests of Immediate Incubation for a Short Time Before Storage as a Means of Sterilizing Contaminated Blood

Since the killing power of blood differs widely between individuals and even in the same person's blood on different occasions, and since some strains of bacteria are more easily killed in blood than others, blood from more than 400 donors and 14 different strains were employed in these tests. Two cold-growing pseudomonas were included which had been isolated from blood believed to have killed two patients. One of them was BP1, already mentioned, the other (BP2) proved to be very similar, although it was isolated in a different part of the country at a different time.

These pseudomonas are actively motile Gram-negative bacilli; they grow readily at room temperature (18–23° C.), slowly at 4° C., and very slowly at 37° C. After one week at room temperature they give the following reactions: citrate positive, methyl-red negative, Voges-Proskauer positive, gelatin liquified, no pigment. They produce acid and a little gas in the following carbohydrate media: glucose, mannite, sucrose, salicin, maltose, sorbitol, glycerol, trehalose and galactose. They produce acid only in lactose and inulin, and they have no action on dulcitol. They are

particularly dangerous because, in addition to growing in refrigerated blood from small inocula within a week, they lyse the red cells very slowly, so that blood appears suitable for transfusion when it is in fact heavily infected.

One strain, BP2, was cultivated in fresh citrated rabbit blood at 4° C. for seven days and then 5 ml. was injected into each of three rabbits. All died within 24 hours, whereas three controls which had received uninfected blood remained healthy, although for some unexplained reason they showed a moderate pyrexia shortly after transfusion.

Before routine incubation treatment can be recommended it must be shown that blood cells are not damaged by it and that even bacteria which grow optimally at 37° C. are incapable of multiplying in the blood during treatment. Twelve hours' incubation as employed in the preliminary test is probably unnecessarily long, because the bactericidal activity of blood against staphylococci is maximal at two hours (Miles and Misra, 1938). In further experiments, therefore, the incubation time was reduced.

Test for Growth During Treatment of Heavily Contaminated Fresh Blood

Eleven freshly isolated strains of *Staph. pyogenes* and one freshly isolated *Bact. coli* strain were employed. The coli was the only strain of six tested which would grow in blood from small inocula.

Small volumes of fresh blood received an inoculum which was equivalent to about 500,000 viable organisms per full bottle of blood for transfusion. In every case the numbers of bacteria after incubation were compared with the numbers in a duplicate refrigerated sample of blood from the same donor.

Method (also employed in a later test).—During routine donation sessions the last 10 ml. of blood from each donor was received into the appropriate volume of citrate in a separate bottle. This was seeded immediately, well mixed, and then divided in half. One sample was refrigerated as soon as possible, in no case later than 30 minutes after bleeding, and the other was treated by incubation at 37° C. for two hours before refrigeration. Sufficient bacteria were added to yield about 10 viable organisms per loopful (0.01 ml.) of infected blood. The cultures were counted by the Miles and Misra (1938) method immediately before seeding the blood and at the end of each experiment. Explants were made from all bottles on to blood agar immediately after seeding, immediately after treatment, and on the following day, when all bottles had been refrigerated overnight.

There was no evidence that any of these 12 strains were able to multiply during two hours' incubation in fresh blood. It seems, therefore, that incubator treatment is harmless even when the blood is heavily contaminated with bacteria which grow optimally at this temperature, but these tests differ from the conditions under which blood is accidentally infected in two important respects. First, the volume of blood is small, so that it cools rapidly, and therefore a comparison between the bactericidal power of small volumes, incubated and refrigerated, favours the incubated sample. In large volumes which cool more slowly in the refrigerator, the blood might be able to reduce the bacterial population during cooling.

The second difference is that, in order to be sure that some viable bacteria were seeded into each small sample, the proportion of bacteria to blood must be high, and corresponds to a very heavy contamination. It is too much to hope that blood can clear itself of unlimited numbers of bacteria, and therefore before proceeding to further experiments using larger volumes and small inocula an attempt was made to estimate the maximal likely contamination during blood donation by the routine method described.

Degree of Accidental Contamination of Blood

Large numbers of bacteria are unlikely to enter the blood unless the citrate is infected or the collecting apparatus has not been cleaned and sterilized. The degree of possible

B

contamination, assuming gross carelessness during collection, was tested by rubbing the moistened rubber wad of the receiving bottle cap with a dirty handkerchief and then rubbing the contaminated wad on blood agar. Fingers of unwashed hands were also pressed on blood agar and the inoculum spread over the surface of the plate to separate conglomerations of bacteria. Colonies were counted after two days' incubation at 37° C. and two days on the bench. The highest count in four experiments with rubber wads was 276 colonies and the highest from single fingerprints of six people was 97 colonies.

Contamination from the air is considered by some to be a serious menace even when blood is collected by a closed method (Braude *et al.*, 1952), but the risk is probably overestimated. The highest bacterial counts in air are found in wards during bed-making, where there may be 2,000 bacteria-carrying particles per cubic foot (70,600 per cubic metre) of air. The total volume of a blood bottle is 600 ml., so that the maximal average contamination would be fewer than 50 particles per bottle if it were deliberately filled with contaminated air immediately before taking the blood. Average counts from crowded rooms are tenfold less than this (M.R.C., 1948). Even allowing that each particle may carry many bacteria and that their distribution in air may be unequal, it is doubtful if in practice more than about 1,000 viable organisms can be accidentally introduced from the air during donation when a closed method is used.

Survival of Small Inocula in Large Volumes of Fresh Blood

Large volumes of blood from 268 donors were seeded with one of four different strains, the two cold-growing pseudomonas, a freshly isolated *Staph. pyogenes*, and the strain of *Bact. coli* which grows comparatively well in blood. The inoculum varied between 7 and 100 viable organisms per half-bottle of blood (approximately 250 ml.) in the different tests. None were made on full bottles because of the need to control each test with an equal volume of refrigerated blood from the same donor.

Method (also employed in a later test).—Two samples of blood, about 250 ml. each, were taken from each donor into bottles containing the appropriate volume of glucose citrate; within five minutes of bleeding each was seeded with liquid culture old enough to have passed the logarithmic phase of growth. Counts were made immediately before and at the end of each experiment. One infected sample from each donor was refrigerated as soon as possible, in no case later than 30 minutes after bleeding; the other bottle of each pair was held at 37° C. for two to six hours before refrigeration.

Bottles seeded with pseudomonas, which grow in the refrigerator, were subcultured at frequent intervals, and if they appeared to be sterile after 28 days they were "incubated" at room temperature for two days and subcultured again as a further test of sterility. Bottles seeded with *Staph. pyogenes* and *Bact. coli*, which do not multiply in blood at 4° C., were tested for sterility after various periods of storage in the refrigerator ranging from 4 to 21 days. This was done to simulate so far as possible the conditions in which accidentally infected blood might be used. Those found sterile on subculture were diluted with an equal volume of peptone water and were reincubated. They were subcultured weekly for four weeks before being finally discarded.

The results (Table II) show that large volumes of blood seeded with small numbers of both cold-growing and warm-growing bacteria are sometimes sterilized when submitted to short-incubation treatment immediately after bleeding, whereas when the bottles are immediately refrigerated this rarely happens. In the fourth test, three of the five samples found sterile without incubation treatment were sterile also in the incubated samples; these may have been very active blood samples. But in two the corresponding incubator-treated sample yielded growth. This may be due to chance, because with such a small inoculum occasional bottles might

TABLE II

Exp.	No. of Donors	Strain	Ability to Grow at 4° C.	Time Stored at 4° C. Before Final Incubation	Inoculum per Bottle 250 ml.	No. of Bottles Found Sterile	
						Treated for 3-6 Hours at 37° C.	Refrigerated Immediately
1	27	BP1	+		14-50	6/27	0/37
2	9	BP1	+		14-59	4/9	0/9
3	80	BP2	+		12-30	1/80	0/80
4	50	<i>Staph. pyogenes</i>	-	15 days	14	23/50	5/50
5	50	<i>Bact. coli</i>	-	21 "	7-14	50/50	49/50
6	50	" "	-	4 "	50-100	49/50	50/50

receive no bacteria. A small proportion of the incubator-treated samples may of course also be sterile by chance, but it cannot account for sterilization of nearly half of them.

In experiments 4, 5, and 6 a time had to be chosen to test survival by incubation, and the results might have been different if the blood had been stored for either a longer or a shorter period. In experiment 4, 15 days was chosen as a time when many bottles of blood are used. When in experiment 5 the bacilli survived in only one of 100 bottles, it was thought possible that prolonged storage (21 days) might have contributed to their death, but in experiment 6 the result was similar in samples seeded much more heavily after storage for only four days.

After treatment one of the pseudomonas was killed much more easily than the other, which suggests that different strains of this organism may have different degrees of resistance to the action of blood.

A marked difference in survival was found between the two human pathogens tested. In experiment 4 the *Staph. pyogenes* survived in 45 of 50 untreated samples, seeded with an average of 14 viable organisms per 250 ml. of blood, whereas in experiment 6 the *Bact. coli* (selected for its ability to grow from small inocula in small volumes of stored blood) died out in all of 50 untreated samples when seeded with 50-100 viable organisms per 250 ml. This may be due to killing by phagocytin (Hirsch, 1956), which was found to be much more active against Gram-negative than against Gram-positive organisms. The effect is different from and completely overshadows the bactericidal action, which depends on temperature. Gram-negative organisms susceptible to it are unlikely to survive in blood at any temperature once the phagocytin has been released.

One further point of interest was that in experiment 3 some samples of the blood which had been incubator-treated yielded growth on storage slightly earlier than the untreated controls. Blood from 30 of the 80 donors which was subcultured almost daily yielded growth on the seventh day from 25/30 of the incubator-treated samples and only 16/30 controls; on the eighth day the figures were respectively 29/30 and 20/30, and by the tenth day growth was found in all bottles.

If many more strains of cold-growing pseudomonas were tested the majority might prove, like BP2, to be resistant to killing by blood. Since it is clear that in any case the method can never be completely effective, further experiments which would involve large quantities of blood are thought to be unjustifiable. If at some later date incubation treatment of blood should seem desirable for other reasons—for example, to activate chemicals added to blood to prolong cell life—further work might be worth while.

4. Effect of Slow Cooling at Room Temperature on the Survival of Contaminating Bacteria

In the preliminary experiments, using small volumes of blood (3 ml.) and the cold-growing pseudomonas BP1, it seemed that on the whole slow cooling was to be preferred to immediate refrigeration after bleeding (Table I). The

test was repeated, using slightly larger volumes of blood (5 ml.), the other pseudomonas (BP2), and one strain of *Staph. pyogenes*. The blood was allowed to remain at room temperature for eight hours before refrigeration, because that is the maximal time likely to elapse between bleeding and storage when the donor session is held at a distance from the centre and there is no refrigerator transport.

Estimation of Growth in Heavily Contaminated Blood During Slow Cooling at Room Temperature

The first method described above (Section 3) was employed. In each case the number of bacteria recovered after eight hours at room temperature (18-23° C) was compared with the number found in a duplicate 5-ml. sample of the same donor's blood which had been refrigerated immediately after inoculation.

One strain of *Staph. pyogenes* did not multiply during this time, but BP2 was able to increase approximately fourfold in fresh blood under these conditions. It would appear from these results that blood infected with BP2 is best refrigerated soon after bleeding; but the conditions of the test differ from those in accidental contamination, as already discussed, in that the blood volumes were small and the inoculum heavy.

The fate of small inocula of both strains of pseudomonas were therefore tested in large volumes (approximately 250 ml.) of blood. The second method described in Section 3 was employed. Half the bottles were left at room temperature for eight hours before refrigeration, and their duplicates were refrigerated as soon as possible, in no case later than 30 minutes after bleeding.

The stored blood was subcultured at intervals for 28 days. Those samples which remained sterile were then subcultured again after 48 hours' "incubation" at room temperature as a further test of sterility.

The two sterile samples were blood from the same donor which was presumably particularly active against BP1.

TABLE III

No. of Donors	Strain	Inoculum per Bottle (250 ml.)	No. of Bottles Found Sterile	
			Treated 18-23° C. for 8 hrs.	Refrigerated 4° C. Immediately
18	BP1	9-46	1/18	1/18
20	BP2	12-30	0/20	0/20

It seems from the results (Table III) that there is little point in early refrigeration, but it was noticed that growth appeared a few days earlier in some of the bottles which had not been immediately refrigerated. Thus, in the first test (BP1) blood from seven donors yielded growth first in the bottles treated at room temperature; blood from one donor yielded it first in the immediately refrigerated bottles; there was no difference in four, and in four the time of growth could not be assessed. In experiment 2 (BP2) the blood from 15 donors yielded growth first in the bottle treated at room temperature, none grew first in the immediately refrigerated bottle, and in five the time of growth could not be assessed. The most frequent difference in time was two to three days, and the maximum noted was four days.

It may appear from this that if growth in some samples can be delayed for a few days, refrigeration might be worth while, but in practice the difference between full bottles refrigerated immediately in a van and those left at room temperature during transport is not as great as in these experiments. The volume of blood was of necessity half a bottle instead of a full bottle, and it was refrigerated in a large cold-room. At a donor session many full bottles are packed into a small refrigerator in the van, and it was found that during transport to the centre they can raise the refrigerator temperature from 4 to 12° C. Probably the advantage of rapid refrigeration in these conditions is so slight as to be negligible.

Inquiries at regional transfusion centres in this country revealed a diversity of practice in that, while centres conveyed blood mainly in refrigerator vans, the time which elapsed between filling the bottle and placing it in the van varied, so that in practice the blood was often allowed to cool slowly for periods up to two hours at room temperature.

Effect of Temperature on Red-cell Survival.—The percentage red-cell survival of incubator-treated and immediately refrigerated blood after 28 days' storage at 4° C. was estimated in four donors using the method of radioactive isotope tagging described by Reeve and Veall (1949) and Mollison and Veall (1955). The results showed a slightly reduced percentage survival time after incubation treatment in all four tests. This is not necessarily a contraindication for incubator treatment, because the test was made late in storage when the percentage survival time is falling rapidly and when, for this and other reasons, blood is very seldom given. If it were tested at 21 days, which is the maximal time blood is normally stored before transfusion, the effect of incubation would probably be negligible.

5. Contamination by Cold-growing Bacteria

It is agreed that bacteria which are able to multiply in stored blood at 4° C. are the most dangerous contaminants (Pittman, 1953; McEntegart, 1956). In Britain during the last ten years approximately 4,400,000 bottles of blood were given and there have been eight deaths thought to be due to contaminated blood (W. d'A. Maycock, 1957, personal communication). Cold-growers were isolated from all of them, and two of these strains were employed in the experiments here recorded.

It was hoped that incubation immediately after blood is withdrawn would effectively kill cold-growers, especially as they are barely capable of growth at 37° C. Although these expectations were fulfilled in some cases, the action of blood is not constant enough to be relied on, and other methods must be sought to prevent contamination or to sterilize the blood.

It has been suggested that the addition of small doses of tetracycline to blood would prevent growth and do no harm to the recipient (Braude *et al.*, 1955). We are not in favour of this, because it is doubtful if all strains of cold-growers are sensitive to any one drug; moreover, antibiotics are not stable, and would therefore have to be added to the bottle after autoclaving, and they are themselves sometimes contaminated. Even very small doses cannot be considered harmless, because they may kill a person who is hypersensitive.

McEntegart (1956) has suggested that danger could be avoided if blood were never stored longer than a week, because his cold-growing strains were not apparent in blood until the second week of storage. Both our strains of pseudomonas, however, yielded heavy growth from small inocula in some samples at six days, and if more had been tested it would probably have occasionally appeared even earlier. By the tenth day heavy growth in all lightly seeded samples was the rule. There is no doubt, however, that the shorter the storage the less chance there is of danger from cold-growers. We also found that if growth had not appeared by the sixteenth day the sample would prove to be sterile.

Prevention of Contamination

We have succeeded in cultivating cold-growing bacilli similar to, but not identical with, BP1 and BP2 from the air, from dust, from swabs of unwashed skin, and in large numbers from fluid in which plastic caps used to cover transfusion bottles are delivered from the manufacturer. The closed techniques in common use could be improved to prevent their entry in the following ways. The bottle should be autoclaved, with the aluminium cap loose, to prevent the formation of a vacuum, and with the holes in the cap covered by adhesive strapping. The strapping should be

removed at the donation session immediately before bleeding and the cap should not be touched before the airway needle (attached to an air-filter tube) and the taking-set needle are plunged through it. When the bottle is full the needles should be removed, still taking care not to touch the cap, which can now be wiped, if necessary, with dry sterile cotton-wool or gauze. The holes should then be sealed with sterile waterproof strapping and the cap covered with an autoclaved plastic cap. The provision of a paper dust-cap over the top of the bottle is not essential, but may be added as a refinement.

There seems to be no reliable way of recognizing infected blood before transfusion. During 1952 to 1955 one London teaching hospital carried out routine stained-film examination of every bottle transfused (15,000), and they came to the conclusion that the risk of infection was so slight (conjectured at 1 in 7,500) that the practice was stopped. Moreover, although the cold-growing bacilli can be seen among the debris when blood is known to be infected and a careful search is made, their presence might well be missed even by experienced workers in a routine examination when blood is urgently needed.

6. Examination of Blood for Infection after Transfusion

Deaths reported after transfusion of infected blood are rapid, and are almost certainly due to foreign substances produced in the blood as a result of bacterial growth rather than to infection by the bacterium. Unless blood is grossly mishandled significant infection by human pathogens which grow at 37° C. but not in the refrigerator is very unlikely. The few staphylococci and diphtheroids which may be introduced from the donor will either die during storage or will remain in very small numbers unless they are given a chance to grow by prolonged incubation or storage at room temperature.

If contamination of blood before transfusion is to be prevented it is important to be able to recognize it when it has occurred. It has become clear to us that revision of culture methods for this purpose is required. Bottles are often taken down at the end of transfusion by someone unskilled in bacteriological technique who is not at the time expecting the remains of the blood in the bottle to be cultured: contamination at this stage is therefore likely. It is necessary to know more about the ability of blood to support the growth of different species of bacteria and the rate at which small numbers of contaminating organisms will grow in it before the most suitable culture methods can be devised and the results of culture can be properly assessed.

Comparison of Undiluted Transfusion Blood and Peptone Water as Culture Media

Comparison of Blood and Peptone Water to Support Growth of Small Inocula.—Growth of 10 to 50 viable organisms in 5 ml. of the two fluids was compared, using a freshly isolated *Staph. pyogenes*, the two cold-growing pseudomonas BP1 and BP2, and stored blood from four donors. In all cases growth appeared earlier and more abundantly in the peptone water. Two further points of interest were noted. The *Staph. pyogenes* grew more readily in old than in fresh blood, and, although the cold-growers flourish at about 20° C. in peptone water, in blood at this temperature their growth is comparatively poor. Small inocula of BP1 died out in some samples of blood at 20° C., although they grew in the same blood at 4° C., almost as well as in the peptone-water controls. Presumably they, like other Gram-negative organisms, are affected by an inhibitor which is active at room temperature and at 37° C., but not in refrigerated blood.

Rate of Growth of Air Contaminants in Blood and Peptone Water at Room Temperature.—Stored blood between 3 and 20 days old from four donors was distributed in 5-ml. amounts in 1-oz. (28-ml.) screw-capped bottles having mouths 1.8 cm. in diameter (transfusion bottles have mouths

2.2 cm. in diameter). An equal number of similar bottles each containing 5 ml. of peptone water were placed in the same racks as controls. Twelve bottles and controls were exposed to air in a ward for seven hours; this included bed-making time, when the air would be very heavily contaminated. A similar set of bottles were exposed for seven hours in the laboratory passage during a time of heavy traffic. After exposure the bottles were closed with sterile caps and left overnight on the bench. The next day and subsequently they were well shaken and subcultured on to two blood-agar plates which were incubated for 48 hours, one at room temperature and the other at 37° C. The results are seen in Table IV.

TABLE IV

	No. of Bottles Yielding Growth at Intervals After Exposure to Air for Seven Hours		
	1st Day	2nd Day	3rd Day
Blood	3/24	8/24	22/24
Peptone water ..	21/24	24/24	24/24

Culture of Blood in Bottles Returned to the Laboratory after Transfusion

Over a period of one month the remains of blood in all transfusion bottles returned to the laboratory were cultured; no transfusion reactions were reported during this time.

The bottles had been sealed after collection and were not opened until used for transfusion. Immediately before use the cap was replaced by the sterile filter and rubber bung of the giving set; blood was not warmed in the bottle. When the transfusion was over, the giving set, which has no air filter, was removed and the cap, which had been exposed to the ward air, was replaced and the bottles were returned to the laboratory, where, for lack of refrigerator space, they stood in the laboratory passage.

The culture method recommended below was employed, but "incubation" at 4° C. and multiple seeding on blood agar were omitted.

Altogether 141 blood residues were cultured between six hours and five days after transfusion. Sterile cultures were obtained from 105 samples, growth in one liquid culture only from eight; growth in both liquid cultures from 17, and growth in all cultures from 10.

The 10 heavily contaminated samples were cultured between one and five days after transfusion. Four of them had been left for one or more days uncapped. (Five of the sterile samples had also remained uncapped for six hours to two days.) One of the remaining six bottles, which was cultured three days after transfusion, yielded a cold-growing bacillus similar to, but not identical with, BP1 and BP2. The others yielded *Staph. albus* (one), *Staph. pyogenes* (two), an aerobic spore-bearing bacillus (one), and one bottle two days old yielded a mixed growth of a spore-bearer and a Gram-negative cocco-bacillus, not a cold-grower. All bottles (16) cultured less than one day after transfusion were sterile.

Other species isolated in liquid culture only were a diphtheroid, a micrococcus, a cold-growing bacillus not identical with BP1 and BP2, a haemolytic streptococcus (not belonging to groups A, B, C, D, or G), and a non-haemolytic streptococcus.

In the light of these results the following method is recommended for the examination of blood when infection is suspected.

Method of Examination of Blood for Infection after Transfusion

After transfusion the used bottles should be capped and returned to the laboratory refrigerator, where they should be kept for at least 24 hours in case investigation is required. When infection is suspected the dregs from the bottles should be cultured even if they have inadvertently remained exposed at room temperature for many hours, because there is a good chance that if the blood was not originally infected it will still be sterile or very lightly contaminated.

The procedure is as follows. Mix the blood well and examine a Gram-stained film for bacteria. Inoculate three blood-agar plates with six separate loopfuls each of the blood and incubate one at 37° C. for two days, one at room temperature (20–25° C.) for one week, and the third at 4° C. for at least as long as the blood had been stored in the refrigerator. (This method of seeding gives a rough estimate of the number of bacteria in the blood. One or two colonies resulting from one loopful might occur by chance, but when each of six loopfuls yields a small number of colonies the blood must be moderately heavily contaminated.)

Inoculate three tubes, containing at least 2 ml. of peptone water or nutrient broth, with five drops each of blood. Incubate one at 37° C., one in the dark at room temperature for a week, and the third at 4° C. for at least as long as the blood had been stored in the refrigerator before the transfusion was given. Return the remainder of the blood to the refrigerator.

Examine the liquid cultures daily, and when growth appears subculture to blood agar and incubate at the appropriate temperature. If after a week there is no evidence of growth, subculture the liquid cultures to blood agar before discarding them and treat the subcultures as already described for the original plate cultures. When growth occurs in only one of the liquid cultures, transfer the others to the favourable temperature to see if they are also infected with the same organism before they are discarded. If no further growth is obtained, make further cultures from the blood which has been kept in the refrigerator, because growth in a single liquid culture may be due to laboratory contamination.

This method is delicate enough to recognize contamination of the order of 5,000 viable organisms per full bottle. When a cold-growing organism is found in profusion—that is, growth on the original blood-agar culture—and damage to the patient can be attributed to no other cause, it should be considered guilty until it can be proved harmless, even if the blood has remained in an uncapped bottle in the ward for 12 hours, because nothing short of injecting a culture into the blood after the transfusion could produce such a heavy growth of this kind of organism in so short a time.

When routine cultures are made of unopened bottles to check aseptic technique during collection it is reasonable to cultivate larger volumes of blood. Since bacteria which contaminate blood are also apt to contaminate cultures in the laboratory, too much weight should not be attached to growth from single liquid cultures. Laboratory technique can be controlled either by making all cultures from the same bottle in triplicate and disregarding less than a two-thirds positive result, or bottles of sterilized nutrient medium can be sampled on every occasion in parallel with the bottles of blood and the infection rate assessed statistically from a large number of cultures.

Discussion

Although some samples of fresh blood incubated for two hours immediately after donation can kill small numbers of contaminating organisms, routine incubator treatment cannot be recommended without further extensive investigation. If it were shown that cold-growing bacilli similar to BP1 were the most likely contaminants during collection and no means could be found to exclude them, routine incubation for a short period would be worth while, because some contaminated bottles would be sterilized and the lives of patients receiving this blood would not be endangered. Most contaminants, however, may be resistant to killing by blood, and if it is collected by the closed method recommended contamination by cold-growers would probably be exceedingly rare. In these circumstances the benefits of incubation might be outweighed by slight damage to the red cells in the numerous uncontaminated samples. In practice, incubator treatment would not be easy to carry out, because some means of automatic refrigeration after two hours would be required.

It has been shown that in practice the transport of blood in non-refrigerator vehicles between donor session and centre was satisfactory, in that over a period of seven years about 500,000 bottles have been so collected with only one fatality due to infection that was caused by an organism which grows in blood at 4° C. Blood contaminated with cold-growers which caused the other seven deaths reported in England and Wales during the last ten years was collected by services which employ refrigerator transport between donor session and centre.

In spite of experimental evidence that some samples of blood contaminated with these organisms will yield growth slightly earlier during storage if not immediately refrigerated, the remoteness of this hazard in practice explains the good results obtained with non-refrigerator vehicles.

Provided that blood can be refrigerated within eight hours of collection there would appear to be no evidence that refrigeration at the collecting session or during transport is essential in temperate climates. Experimental incubation of blood for two hours immediately after collection suggests that in the tropics it will come to no harm if left for at least two hours unrefrigerated.

It seems that one reason why accidents from infected blood are not more common may be that blood for transfusion is a poor culture medium.

Summary

The problem of contamination of blood for transfusion was investigated, particularly with regard to the effect of temperature after collection on contaminating organisms.

Bacteria which grow at 37° C. and two strains isolated from infected blood which grow at 4° C. were inoculated into samples of freshly drawn blood in an attempt to reproduce the actual mode of infection during donation.

It was demonstrated that, although experimentally there are slight advantages in refrigerating blood within 30 minutes of collection, in practice refrigeration in temperate climates is not essential until at least eight hours after collection.

Some samples of blood held at 37° C. for two hours immediately after donation killed small numbers of contaminating bacteria; none of the strains tested multiplied in blood during this time.

Prevention of contamination by cold-growing bacteria is discussed and a method is recommended for the examination of blood suspected of infection.

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ADDENDUM.—Since this paper was completed, results of experiments on the survival and growth of bacteria in bank blood incubated at 37° C. for short periods before refrigeration have been published by Geller, P., Chandler, L., and Jawetz, E., *J. Lab. clin. Med.*, 1957, 49, 573. So far as the results can be compared, they are in agreement.

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SPLENECTOMY IN ACQUIRED GENERALIZED MYELOSCLEROSIS

BY

N. K. SHINTON, M.B., M.R.C.P.

Senior Registrar in Clinical Pathology, Queen Elizabeth
Hospital Birmingham

Acquired generalized myelosclerosis with associated myeloid metaplasia is an uncommon but well-recognized disorder which has been described under a variety of names, including osteosclerotic anaemia, myelophthisic anaemia, megakaryocytic myelosis, and agnogenic myeloid metaplasia. It is regarded as distinct from the hereditary osteopetrosis or marble bone disease of Albers-Schönberg, which occurs in childhood and young adults. Acquired myelosclerosis is not familial, and is seen in patients of both sexes more commonly after the age of 40. The clinical features are an insidious progressive anaemia, usually of leuco-erythroblastic type, and gross splenomegaly. The anaemia may later show a haemolytic component or may be associated with neutropenia or thrombocytopenia. Radiographs of the bones sometimes show mottled rarefaction or irregular condensations in the cortical portions and splintering or elevations of the periosteum (Rosenthal and Erf, 1943). The prognosis is poor, death occurring usually within five years from cardiac failure, intercurrent infection, or haemorrhage. At necropsy there is replacement of the bone marrow by fibrous or osseous tissue and extramedullary haemopoiesis, myeloid metaplasia occurring in the spleen, in the liver, and sometimes in the kidneys and lymph nodes.

Aetiology

The cause of the condition remains uncertain, but in a few cases it has been reported in association with carcinomatous deposits in the marrow, myeloma, Hodgkin's disease, and diffuse tuberculous infection. Wyatt and Sommers (1950) reviewed a group in which they suggested that the changes were a result of intoxication by various organic chemicals. Donhauser (1908) was the first to regard the myeloid metaplasia as a compensatory extramedullary haemopoiesis and the spleen as the most important source of red cells, granulocytes, and platelets. If this is a correct hypothesis, splenectomy would be clearly contraindicated (Scott, 1949; Wintrobe, 1951; Learmonth, 1951; Chatterjee, Meza Arrau, and Dameshek, 1952; Hickling, 1953).

An alternative hypothesis supported by Vaughan and Harrison (1939), Dameshek (1951), Hutt, Pinniger, and Wetherley-Mein (1953), and Richardson and Pinniger (1953) regards the myelosclerosis and the myeloid metaplasia as part of a proliferative disorder of mesenchymal tissue, in response to an as yet unknown stimulus. As the mesenchymal cell is multipotential and may differentiate into a fibroblast, erythroblast, myeloblast, or megakaryoblast, the clinical and haematological pattern depends upon the predominant cell group and the stage of development involved. Myelosclerosis has been described in association with polycythaemia vera in a number of cases reviewed by Beattie and Withey (1953), since when further reports have been added by Robson (1953) and Korst, Clatanoff, and Schilling (1956). Cases of myeloid leukaemia with myelosclerosis have been reported by Hutt *et al.* (1953) and Richardson and Pinniger (1953).

Richardson and Pinniger (1953), Croft (1956), and Asher (personal communication) describe cases where the features of myelosclerosis, polycythaemia vera, and leukaemia have occurred in the same patient at different times, as has also megaloblastic anaemia in the case described by Croft (1956). According to this myeloproliferative theory, the myeloid