BACTERIOLOGICAL CONTROL IN A BLOOD BANK: A REPORT

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AMONG THE AUTHORS who have studied bacterial contaminants of blood, Braude (1) reports contamination by gram-positive and gram-negative microbes. The former comprise pathogenic staphylococci and diphtheroids which do not cause serious reactions in man. The latter are much more dangerous and are known to be responsible for severe shocks on account of their endotoxins (2, 3, 4). As stated by Braude, Corey, and Siemienski (5) these gram-negative germs can be divided into two groups according to their physiology:

1. A first group comprises saprophytic species belonging to the genera *Pseudomonas* and *Achromobacter*. These bacilli are cryophillic: their optimum temperature being between 4° and 8° C.; when incubated at 37° C., the growth is very poor and slow to develop.

2. A second group contains microbes of the coli-aerogenes group: *E. coli*, *E. freundii*, *E. intermedium*. Pittman (6) reports other gram-positive contaminants such as: streptococci, sarcina, and *B. cereus*. He also cites blood contamination by *Paracolon intermedium*, *Paracolon aerogenoides*, *Faecalis alcaligenes*, and other germs capable of utilizing citrate as the sole source of carbon. In eighteen fatal transfusions, he has found eight strains of the genus *Pseudomonas*. However, *Pseudomonas aeruginosa* was not involved in these accidents.

According to Braude (1), the rate of contamination in blood banks is between 2 and 3 per cent. Reactions are rare and occur only when blood is heavily contaminated. In England, during the war, 1939 to 1945, blood contamination in blood banks was about 5 per cent according to Whilly (7), while in New York, during the same period, Heath and Angrem (8) reported an 8.5 per cent contamination calculated on a quantity of 6,151 L. blood. Walter, Kundsin, and Button (9) report 47 contaminations from 4,497 specimens (1.04 per cent) in which were found *Staph. aureus*, streptococci, a flavobacterium, yeasts, aspergilli, and penicillium.

The reason why slight contamination is not very dangerous is that fresh blood has antibacterial properties apparently related to the presence of antibodies and complement as pointed out by Geller, Chandler and Janetz (10), also of properdin according to Wardlay and Pillemer (11). These properties seem to be enhanced by gram-negative microbes following a short period of incubation at 37° C., before the storage of the blood in the bank; this activity is not so evident for cryophillic bacteria which later multiply in the refrigerator. Low temperatures are believed to inhibit these antibacterial factors. This present paper deals with the search for bacterial contaminants in a normally operated blood bank. Its aim was to control the sterility of the blood and to compare methods and results with the data from the above-mentionned authors.

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MATERIAL AND TECHNIQUE

The samples were taken from the blood bank of the Hôtel-Dieu Hospital in Quebec City during October 1958, the blood having been kept in the refrigerator at 6° to 8° C. for periods varying from two to 31 days. By preference, the oldest specimens were chosen so that even the slowest growing organisms such as spores, fungi, or cryophillic bacteria might be detected. Blood specimens were taken from the containers (rubber caps well cleaned with 70° alcohol) in a most aseptic manner by the anaesthetist and the bacteriologist assisted by a nurse, all three wearing masks. 2.5 ml. were withdrawn and inoculated immediately into a test tube containing 7.5 ml. thioglycollate liquid medium.[3] (Thioglycollate medium has long been recommended for sterility tests; it may be used alone, with methylene blue or even better still with resazurin as an rh indicator, Pittman (12)). The last drop of the blood in the syringe was used for a direct examination. In this way, 58 specimens were collected; of these, 30 were taken from glass containers,[4] the remaining from plastic bags.[5] All specimens were incubated for one month: 15 days at 37° C. and 15 days at room temperature (20°).

Following this incubation period, a second direct examination was made for all tubes and a gram staining was done whenever necessary. May we stress the necessity of making direct examination, because apparently one cannot rely on the clarity of the culture media to conclude that they are sterile; as a matter of fact, Wichelhausen, Clark, Griffing, and Robinson (13) have shown that a solution of human serum-albumin diluted to 25 per cent may be quite contaminated without showing any turbidity. These authors have thus isolated from

TABLE I

Number of cases	Storage times (days) at 6 to 8°C.	Results obtained after 15 days at 37°C. and 15 days at 20°C.
1	31	Both direct examinations and cultures were negative.
1	30	
3	23	
2	21	
1	20	
4	19	
1	17	
3	16	
3	15	
3	13	
2	11	
3	10	
5	9	
12	8	
2	7	Three specimens gave a culture of *Faecalis alcaligenes*.
3	6	
4	5	Both direct examinations and cultures were negative.
5	2	
58		

[3]No 01-140, Baltimore Biological Laboratory Inc.
[4]Transfuso-vac., F. 83, Baxter Laboratories of Canada, Acton, Ont.
[5]Pliapak A.D.C. Solution, Abbott Laboratories, Montreal.

clear sera the following organisms: *C. albicans, Proteus vulgaris, P. aeruginosa,* and *Streptococcus faecalis.*

Table I summarizes the procedures followed and also the results.

Results and Discussion

Only three specimens of blood from glass containers gave positive cultures (*Faecalis alcaligenes*) after 25 days of incubation. It is most probable that these specimens were contaminated in the laboratory, as: (i) the cultures were examined macroscopically every day, and turbidity appeared only on the twenty-fifth day, after 15 days of incubation at 37° C., and 10 days at 20° C.; (ii) the plasma haemoglobin content of the three specimens of blood was very low; (iii) the patients who received the blood presented no reaction whatever.

These contaminations have undoubtedly occurred through the cotten plugs of the culture tubes. In order to prevent such casualties, it may be advisable to seal the tubes or to use rubber caps. According to Marfarlane, Mainwaring, Maesween, and Parish (14), it is most difficult to avoid contamination when cultures have been inoculated for a long period of time, a... in order to overcome this difficulty they described a method for sealing the tubes.

As noted previously, only massive contaminations cause serious post-transfusion accidents. It may be of interest to recall briefly that reactions occur thirty minutes or more after the transfusions. The symptoms are chills, temperature, muscular algia, hypotension, and shock which may be fatal (15–17). As stated by Braude (1), such accidents can be prevented by taking the following precautions: (i) a direct examination of the blood immediately before the transfusion; (ii) a routine use of 20 mg. of tetracycline per litre of blood. Braude recommends these precautions when storage conditions are poor, or when difficult manipulations are inevitable as in wartime or during a civil catastrophe.

In order to prevent an eventual multiplication of microbes, the time lapse between the collection of the blood and the transfusion should be as short as possible. According to Braude (1), the bacterial multiplication may reach important proportions in a week or two, in such a manner as to give concentrations of 10^9 to 10^{11} germs per millilitre.

We believe that whenever procedures of collecting blood have been long and difficult, the laboratory should proceed to immediate cultures and that such blood should be used within a week. A direct examination and a gram staining made immediately before the transfusion are advised. Stevens, Legg, Henry, Dille, Kerby, and Finch (4) recommend a staining just before transfusion. According to Walter *et al.* (9), a sterility test should be made before a transfusion, whenever the blood has been refrigerated for more than 96 hours. These same authors also describe a new technique for the detection of blood contaminants by the use of plastic equipment which seems to lessen the risks of contamination, in comparison to glass containers.

Conclusion

After 25 days of incubation three blood bank specimens out of 58 were con-

taminated with *Faecalis alcaligenes*. These contaminations occurred in the laboratory, as the other 55 samples remained sterile even after one month of incubation. This was made evident by a gram stain of the cultures.

In a well-organized blood bank, contamination can be avoided, at least in blood specimens used for transfusions, by having this service conducted under the supervision of an anesthetist working in close co-operation with the laboratory staff.

SUMMARY

Fifty-eight specimens of blood from a blood bank were inoculated in fluid thioglycollate, and incubated 15 days at 37° C., and 15 days at 20° C., for sterility control; only three yielded a culture of *Faecalis alcaligenes* on the twenty-fifth day of the experiment, and it is assumed that this was caused by a contamination in the laboratory.

A general review of the subject is given, along with a discussion of the prophylaxis of blood bank contamination.

RÉSUMÉ

Cinquante-huit spécimens venant d'une banque de sang ont été ensemencés dans du thioglycollate liquide et incubés durant 15 jours à 37° C., et durant 15 autres jours à 20° C., pour contrôle bactériologique. Trois échantillons ont donné une culture de *Faecalis alcaligenes* au vingtième jour de l'expérience; selon toute évidence, il s'agissait d'une contamination survenue au laboratoire.

Une revue générale de la question est exposée, ainsi que les moyens à prendre pour prévenir la contamination du sang des banques.

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