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It is generally known that after some transfusions of blood and blood derivatives reactions of various kinds and seriousness follow. They are mostly pyretic, allergic, hemolytic, coronary and others. Among the feared, but relatively rarely occurring reactions are those from bacterially contaminated blood.

Bacterial contamination may come about:

1. Through incorrect preparation of the transfusion material, such as the withdrawal equipment, transfusion flasks, preservative solution, and, particularly, through inadequate sterilization;
2. During the withdrawal of the blood itself (bacteriemia of the donor, contamination of the withdrawal syringe with the microbic flora of the skin in case of poor antisepsis of the donor's skin, or of the tip of the syringe by airborne bacterial flora);
3. During the storing of blood (mainly defects of the sealing or puncturing of the flask);
4. During the transfusion (mainly through contamination of transfusion equipment).

The presence of microbes in the blood container may show in vitro either by hemolysis (Braude and coworkers estimate that 75% of psychrophilic microbes quickly cause hemolysis), or by sedimentation as result of the utilization of the citrate: 18% of the microbes. A considerable amount of microbes can be also sometimes shown macroscopically through milky clouding of the plasma. A drop of the osmotic resistance of erythrocytes may be reason to suspect bacterial contamination.

It is understandable that even in the case of bacterial contamination mesophilic microbes will not multiply if the container is properly stored in a refrigerator with a temperature of +2 to +4°C as ordered in the directives of the NTS (National Transfusion Service). However, even at this temperature some psychrophilic or psychrotolerant microbes can multiply quite well; some moulds also multiply

well at a temperature of +2 to +4°C.

Some authors say that in bacterial contamination multiplying of gram-negative psychrophilic microbes does not take place immediately, but only after an initial latency period which last 3-4 days (Entegart), according to others, seven days (Geller and coworkers). Then begins the live division of microbes (Entegart): three times in 24 hours.

The microbic flora in the blood preparation can be demonstrated either with a stained smear or with a culture. According to Chaplin and coworkers, bacterial contamination can be proven with a culture in 24 hours if there are at least 24 microbes per millilitre of blood. According to the same author, the same proof can reliably be made with a smear only if there are at least 240,000 microbes per ml of blood.

The seriousness of post-transfusion reactions after transfusions with contaminated blood justifies the need of a thorough bacteriological control system at the transfusion stations. The extent of the bacteriological control is exactly determined by the directives of the National Transfusion Service, which are binding for all transfusion stations in our state.

Technique and Method of Bacteriological Control

The bacteriological control was carried out in our institute exactly according to the directives which in the given time determined the method and technique of bacteriological investigations. One may say that the system of bacteriological control has been steadily broadened during these years, although the technique has not basically changed.

The bacteriological control carried out in our institute in accordance with the nation-wide practice of the NTS concerns: 1. control of the resulting therapeutic product, i.e. the blood, erythromase, erythrocyte resuspence, plasma, etc.; 2. all activity at the transfusion station is subject to bacterial control, including 3. control of carrier personnel.

Of all the transfusion products, most attention is received by plasma, because the amount of handling in its preparation is substantially higher than with other products. In plasma control we make, in additional, cultures from remains of plasma in large distribution bottles. We start from the assumption that microorganisms may settle on erythrocytes, which then gradually sink to the bottom of the bottle. By using cultures of the sediment we avoid the possibility of overlooking positive results in plasma samples drawn from the upper part of the bottle in which there is only the bacteriologically contaminated sediment. From the sediment we also prepare stained smears for microscopic examination. The making of smears is important to show the massive bacterial contamination in which the multiplying of microbes was so large that it results in the death of these microbes because of complete consumption of the nutrient substratum. One might in such a case obtain through the culture method

Table 1.

Percentages of Bacterial Contamination of Transfusion Material in the Institute for Hematology and Blood Transfusion, 1952-58.

Kind of Bacterial Control	1952 %	1953 %	1954 %	1955 %	1956 %	1957 %	1958 %
Transfusion preparations:							
Plasma	5.2	0.31	0.58	0	0	0	0.26
Plasma-sediment	2.07	0.25	0.72	0.19	0	0	0.52
Erythromase	10.8	0.41	0	0	0.27	0	2.8
Resuspense	1.8	0.5	0	0	0	0	0
Control Blood	11.0	0.4	0	0	0	0	0.9
Blood withdrawal in Booth:							
Smears from Arms of Booth Personnel	87.0	59.0	54.0	39.0	44.0	34.0	27.0
Smear from Arm of Donor after Disinfection	21.0	22.0	11.0	19.0	18.0	22.0	14.0
Gloves of Booth Personnel	60.0	30.0	36.0	21.0	31.0	13.0	10.1
Surface of Flasks	58.0	22.0	5.5	12.0	24.0	31.0	26.0
Necks of Flasks	0	16.0	8.2	10.2	15.0	86.0	19.0
Surface of other sterile withdrawal Equipment	17.0	10.0	88.0	15.0	17.0	13.0	10.0
Control of Activity:							
Preservative Solution, before Sterilization	3.2	5.0	1.0	0	9.0	22.0	9.4
Solutions after Steriliz.	0	0	0	0	0	0	0
Autoclaves	0	0	0.4	0	0	0.4	0
Nonpyrogenic Apparatus	51.2	54.0	27.0	34.0	53.0	10.0	24.0
Washroom	57.0	56.0	56.0	75.0	74.0	64.0	47.0
Montage	18.0	28.0	36.0	55.0	57.0	56.0	76.0

Table 2

Percentage of Incidence of Bacterial Contamination of Blood Preparations Abroad

Author	Year of Publication	% of Bacterial Contamination
Macfarlane	1942	5,0—25,00
Braude	1952	2,27
Heilmeyer	1953	11,4
Petzelt	1953	4
Stevens	1953	1—3
Vonkilch	1954	9—15
Bergmann	1954	4,1—11,7
Discombe	1954	3—5
Braude	1955	2,2
Chaplin	1956	3,87
Pettenkofer	1957	1—5
Walter	1957	1,0—6,01
James	1958	0,25
Bonnel	1958	6,0
Gibson	1958	1,6

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the wrong finding of bacteriological sterility.

The control of the blood preparation is done at random in this way: During withdrawal of the donor s blood two small samples of blood are drawn into control bottles which contain normal autoclaved preservative solution. If then both control bottles are sterile, we assume that both the preparation of the preservative solutions and the withdrawal itself was done in a sterile way. The control of plasma is done by bacteriologically controlling one plasma sample from each production run. In addition a random bacteriological control is carried out on one percent of all remaining erythromase or resuspense.

With regard to the prevention of pyretic reactions, we also control the thoroughly cleaned material before placing it in the autoclave. We are conscious of the fact that although the material may be sterile, the number of microbes on the transfusion equipment should be as small as possible before autoclaving, since even dead microbes at autoclaving, if their number exceeds the fever-causing dose, may also cause a post-transfusion reaction; therefore the employees in the washrooms must try to keep microbic contamination of transfusion equipment as small as possible. For this purpose steady bacteriological control of the work is necessary.

The bacteriological control of water for the making of preservative solutions and for washing of transfusion materials is also systematically complemented with biological control of pyrogenes. The demand for sterilization of the preservative solution up to two hours in the autoclave after its preparation aims at prevention of multiplication of microorganisms and thus of pyretic reactions. We determine the number of microbes present in preservative solutions before placing them in the autoclave. If it contains more than 20 germs per ml of solution (the orientation method of pouring two ml of solution on a Petri dish cooled with agar is used) it is necessary to find the bacteriological originator of the impurity (chemicals, water, air, poor work habits).

Results

The extent of the bacteriological control carried out in our station and its results are summarized in Table 1. which also gives the percentage of the incidence of bacterial contamination of the various kinds of transfusion materials, and according to years from 1952 to the present. There are listed the results in percent of the contamination of end products, i.e. blood, erythromase resuspense and plasma, and the results gained by control of the activity in the transfusion station of the institute (control of sterility, preservative solutions before autoclaving, control of the functioning of nonpyrogenic aparatus and autoclave, control of sterility by means of smears from the hands of personnel from the withdrawal department with surgical scrubbing, control of sterility by means of smears from the arms of donors before blood withdrawal, control of sterility of

rubber gloves, control of the sterility of the surface and necks of the transfusion flasks, control of the sterility of the other withdrawal equipment, etc.).

Discussion

In Table 1 is above all shown the steady drop in percentage bacterial contamination in recent years. This drop is caused both by the higher quality of work in the transfusion station and better training of workers at the station during the past seven years of the existence of the transfusion center at the Institute, mainly through the thorough application of demands of strict asepsis and sterility in blood taking in sterile booths (as compared with earlier blood taking in the field).

The seemingly paradoxical increase of bacterial contamination in 1958 can be explained by the fact that in that year the work plan dropped at the transfusion center of the institute through a decrease of routine work, so that the percentages are computed from a small number of sterility samples compared with number of examinations undertaken in previous years.

The sterility of our transfusion preparation stands out particularly in comparison with the percentage figures on bacterial contamination of blood preparations given in the foreign literature (see Table 2.).

The conspicuous differences appearing in this table may be explained also with the varying levels and working methods of the transfusion services in the various countries. Considering the fact that overall care in blood transfusion is rising, the works from more recent years show on the average a lower percentage of contamination than before. A noticeable improvement came with the use of plastic transfusion bags (blood preparation in glass bottles: percent contamination 6.01, contamination in plastic bags 1.04% -- Walter and coworkers 1957 /547). Some differences may also be explained by different methods of bacteriological investigation (a single examination of blood preparations gave 4.1% positive findings, a repeat examination of the same preparations, 11.7% positive contaminated preparations -- Bergman 1954). If used for cultivation of preparation after use for transfusion in treatment departments, the percentage of contaminated preparations rises considerably (Chaplin).

In order to prevent the multiplying of microbes, antiseptics were added to the blood preparations (for instance, rivanol), sulfonamides (here earlier for instance alesten), salicyl, urotropin (Schurch), more recently antibiotics also (streptomycin in the USSR, in the US tetracyclin, for instance, Braude et al., 1955). It can be said, however, that the protective effect of these substances is inadequate. The instability of antibiotics in autoclaving is also a disadvantage and also the possibility of fatal reactions in intravenous administration of even a small amount of an antibiotic to hyperegic individuals (James et al.).

Table 3.

Differential Diagnosis and Treatment of Post-Transfusion Reactions caused by the Transfer of Bacterially Contaminated Blood.

Kinds of Microbes	Gram + saprophytes	Microbes growing in cold (pseudomonas or achromobacterium)	Coliform bacilli *)
Diagnosis			
Smears from transfused blood	+	+	+
Smears from pulmonary blood	±	±	±
Culture from transfused blood at room temp. 27°C	+	+	+
Culture of Patient's blood	(?)	0	+
Elevated temperature	+	+	+
Muscle pains	0	+	+
Drop TK	0	+	+
Diarrhea	0	+	+
Treatment			
Various antibiotics	0	0	+
Adrenalin	0	+	+
Cortisone	0	+ (?)	+ (?)

*) Citrate positive, usually paracolon bacillus

From a clinical point of view the transfusion of contaminated blood can have reactions of varying seriousness. Transfer of mildly contaminated blood leads to a pyretic reaction. One can observe in gross bacterial contamination the symptoms of fulminant septicemia. A serious or even fatal course may follow after contamination even with microbes non-pathogenic for man, since they create in the preparation toxic products with a strong hypotensive effect. This applies particularly to some gram-negative psychrophylic microbes. For instance, the polysaccharid from Serratia marcescens, the pyrogen from Pseudomonas aeruginosa and others have a strong

hypotensive effect. (Braude and coworkers). Stevens and coworkers states that gram-negative microbes are on the average 100 times more pyrogenic than gram-positive microbes. Almost all fatalities described in the literature after transfusion of contaminated blood were caused by gram-negative microbes.

Clinically the reaction after blood transfer has several phases (Stevens).

1. During the podromal stage (30 minutes after the beginning of the transfusion) headache, restlessness, and tremor appear.

2. During the second state (30-60 minutes after the beginning of the transfusion) sudden chills appear, which last 10-30 minutes and are followed by a steep rise in temperature.

3. The third phase is characterized by extreme distension of the arteries well visible in the mucous membranes, warm skin, gastrointestinal disturbances (nausea, vomitting, diarrhea) and sudden drop of pressure. These symptoms appear about 60 minutes after the beginning of the transfusion. For this type of post-transfer reaction violent abdominal pains and skeletal muscle pains on pressure are also characteristic. (Baude). The cause of the abdominal pains is the hyperperistalsis of the intestines, pains of the skeletal musculature are caused by increased muscular rigidity.

4. The fourth phase is of shock, disappearance of vasomotor regulation, strong orthostatic hypotension with peripheral accumulation of blood (duration: 1-6 hours). Death occurs in 50-65% of the cases (Stevens).

Baude and coworkers (9) recommend antibiotics therapeutically and in the shock phase, adrenalin and cortisone.

The differential diagnosis and treatment after transfer of contaminated blood is outlined in summary form in Table 3 (according to Braude).

Table 4.

Percentage of all Post-transfusion Reactions in the Territory of our State in the Period 1950-1958.

	1950	1951	1952	1953	1954	1955	1956	1957	1958
Reactions after whole blood	5.2	2.8	1.9	0.4	0.7	0.6	0.6	0.5	0.5
Reactions after plasma	1.5	0.8	0.4	1.2	1.0	0.6	0.7	0.6	0.5
Reactions after erythrocytic sediment	not made	not made	1.3	1.2	1.0	0.6	0.7	0.6	0.5

As far as the kinds of microbes are concerned, according to our experience sporulating microbes are a sign of dust in the environment. Frequent incidence means that more attention has to be

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	1950	1951	1952	1953	1954	1955	1956	1957	1958
USSR		5% Kizilova	10,64% Sokolova		3—5% Petrovskiy	7,2% Belenskiy			
USA		6,8% Scudder 5,2% Lundy	7,1%						2—8% Discombe
England									2,6—4,5% Mayer
France									8% Ringenbach
Germany			10% Heim		4,4% Busch	1—2,5% Reissigl		3,7% Lau	
Poland				8,7% Plachecka					
			5,8% Judkiewicz						
Hungary		14,08% Verecki			2,5% Balint				
Austria		4% Fuchsig							
Netherlands	9,6%	3,7% Stallman	3,7%						
Switzerland				6,9% David					
Sweden						5,96% Ramgren			

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Table 5

Percentage of Reactions after Transfusions of whole Blood abroad according to various Authors

given to cleanliness, or, if necessary, to disinfection. Gram-negative microbes have been most often obtained from water. They got into the transfusion preparation either through failure of the autoclave or from the washed hands of employees. Gram-positive cocci are usually of skin origin and are transferred to transfusion materials by unnecessary touch.

Regular smears from donors' arms forces the personnel of the department to clean the arms properly; this decreases the possibility of infection from skin microbes, and assures also the safety of the intravenous injection for the donor. The other controls in the withdrawal and plasma aim at booth prevention of infection of the transfusion preparations, like, for instance, the sterility control of the hands of the personnel after scrubbing before working the booth starts, sterility control of rubber gloves of workers in sterile booths, control of the surface of the bottles with stored blood brought into the booths for drawing off plasma, etc. All these measures force the workers in these departments to the greatest care for the asepsis and sterility of all work, and the bacteriologist becomes the living "conscience" of the workers in the transfusion station.

As far as the particular directives for the Czechoslovak transfusion service are concerned, a similar system of bacteriological control as in this institute is strictly followed in all transfusion stations. The systematic care for the sterility of transfusion products is one of the reasons for the very low percentage of post-transfusion reactions in our country, and here also there is a steady decrease in the percentage of reactions from 1950 until now, as can be easily seen in Table 4.

The low percentage of post-transfusion reactions here testifies to the very high state-wide level of the Czechoslovak Transfusion Service with respect to the safety of blood transfusions, and it stands out especially in comparison with the percentage of post-transfusion reactions in some other countries, which are summarized in Table 5.

We wish to thank Dr Edward Dobry of our institute for kindly making the percentages of state-wide post-transfusion reactions available for Table 5.
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Summary

The extent of bacteriological controls in the Czechoslovak Transfusion Service and its importance in the prevention of post-transfusion pyretic reactions following transfusion of bacterially contaminated blood is reviewed. Results of control examinations for bacterial sterility of transfusion preparations and activities in the transfusion station, obtained by us in our institute during 1950-1958, are summarized. Comparison with results from abroad shows that the standard of work is excellent with regard to bacteriological sterility and safety of blood transfusions.

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