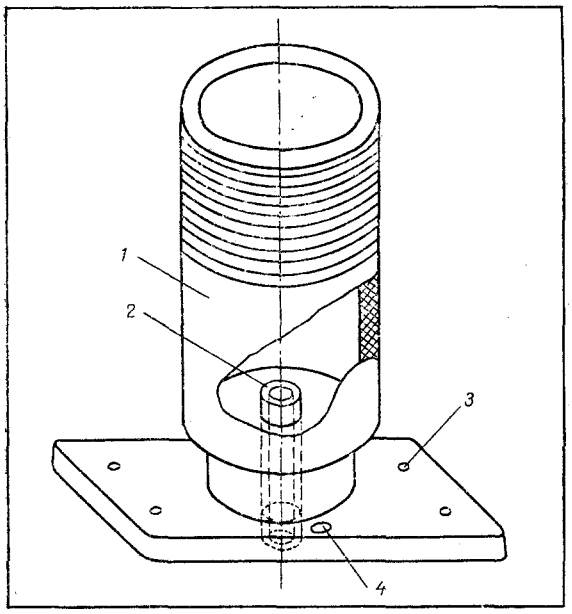

Fig. 1. Design of the removable fistula: 1) outer part of fistula with thread for lid; 2) length of silicone tube through which the leads are taken; 3) holes for suturing fistula to abdominal wall; holes into which omentum can grow (reduced in size).

possible; 3) nowhere are there any rigid fixation points, which is where the leads usually break; 4) exteriorization of the leads through the abdominal wall creates a barrier (the omentum) to the spread of infection and excludes the development of inflammation in the chest almost entirely; 5) the method of making airtight the place where the leads are brought out in the fistula means that they can be used many times; 6) if necessary, leads from sensors implanted on the abdominal organs can be brought out through the same fistula.

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TECHNIQUES FOR LONG-TERM STORAGE OF HUMAN LYMPHOCYTES

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When lymphocytes stored in the frozen state are used in the lymphocytotoxic test the fraction of cells dying after thawing must not exceed 10-20%. The effect of various concentrations of dimethyl sulfoxide (DMSO), the composition of the medium, and other parameters accordingly was investigated in order to work out optimal conditions for freezing human lymphocytes. The best results were obtained by freezing the cells in autologous serum with 12.5% DMSO. However, sufficiently good results also were obtained by the use of AB serum and 20% bovine serum in Eagle's medium.

KEY WORDS: human lymphocytes; freezing.

When human leukocytes are studied in order to type them with respect to the various antigen systems, in the blast-transformation reaction, and for other purposes the need often arises for a repeated or more detailed investigation. However, it is not always possible to obtain

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TABLE 1. Number of Dying Lymphocytes (in %) after Freezing in Hanks's Solution with Autologous Serum and DMSO

Autologous serum (in %)	DMSO (in %)						
	0	5	10	12,5	15	17,5	20
3	—	57	31±6	37±6	44±6	66±15	66±12
n		1	8	7	5	3	3
43	86±3	—	12±2	10±2	11±1	14±2	31±9
n	20		7	8	5	3	3

Legend. Here and in Tables 2 and 3, n indicates number of tests.

TABLE 2. Results of the Use of Different Media for Freezing Lymphocytes

Index	Serum			Serum albumin						
				bovine						human
				British		Soviet				
	autologous	AB 100/40	bovine 100/40	3/1	3/1	7/3	10/4	12,5/5	15/6	3'1
Number of cells (in %) preserved after thawing	48±4	60±7	53±10	—	—	—	57±6	64±3	64	—
n	10	8	4				8	5	1	
Number of dying lymphocytes (in %)	12±1	16±2	19±3	45±13	21±3	23±3	17±2	19±3	22	51±14
n	19	10	4	5	8	7	9	6	1	3

Legend. Fractions give concentration of corresponding medium (in %): Numerator shows initial and denominator final concentration. All media made up in Hanks's solution with autologous serum (3-10%). Concentration of DMSO 12.5%.

TABLE 3. Comparative Results of the Use of Undiluted Autologous and Bovine Sera for Freezing Lymphocytes

Index	Serum			
	autologous		bovine	
	DMSO (in %)			
	12,5	5	10	12,5
Number of cells (in %) preserved after thawing	57±5	29±8	42±14	50±5
n	9	5	3	13
Number of dying lymphocytes (in %)	13±1	28±7	16±3	16±2
n	5	5	3	10

another blood sample from the person who is being tested, and in some cases this is absolutely ruled out. Methods of prolonged storage of isolated leukocytes [1, 3, 4, 6, 7] have accordingly been suggested, based on gradual freezing of the cells to −40 to −50°C in the presence of cryoprotectors, followed by storage at between −80 and −196°C. The cryoprotector usually used is dimethyl sulfoxide (DMSO), the advantages of which over glycerol have been demonstrated by Dougherty [2], but the details of the procedure (the concentration of DMSO, composition of the medium, and so on) vary considerably. The problem is made still more difficult by

the fact that when unfrozen leukocytes are used for typing and in the lymphocytotoxic test the fraction of dying cells must not exceed 10-20%. It was therefore decided to investigate the optimal conditions for freezing lymphocytes.

Cells were isolated from the venous blood of healthy human donors in an Isopac—Ficoll gradient and the lymphocytotoxic test was carried out by the method of Kissmeyer-Nielsen and Thorsby [5]. The lymphocytes were allowed to stand overnight in Hanks's solution with autologous serum (25-33%) at 4°C, resuspended in the test medium with DMSO ($1 \cdot 10^6$-$3 \cdot 10^6$ cells/ml), cooled to 4°C, and frozen in a special apparatus at the rate of 1 deg/min to −40°, and thereafter at the rate of 5 deg/min. The cells were kept in liquid nitrogen. They were thawed in a waterbath (40°C) for 1 min, if frozen in special plastic tubes, and for 20 sec in ordinary cocktail straws. The lymphocytes were washed with Hanks's solution, resuspended in the test medium and, after appropriate incubation, the number of cells preserved was determined (in % of the number frozen), and the number of these cells which had died was counted (in % of 200 cells) by the use of trypan blue. Altogether more than 42 different methods of freezing were studied.

In the experiments of series I various concentrations of DMSO were tested (Table 1). The best results were obtained with a concentration of 12.5%, but in the presence of a considerable amount of autologous serum. Since an extra amount of blood has to be taken in order to obtain this serum, in the experiments of series II the following media were tested: group AB serum (without preservative), inactivated bovine serum and human serum albumin (West Germany), and bovine serum, of either British (Light and Co.) or Soviet (Mark B, Olainskii Factory) manufacture. The results are given in Table 2. They show that although autologous serum gave the best results, sufficiently good results also were given by albumin from the Olainskii Factory in a final concentration of 4% and by AB and bovine sera. Since bovine serum is readily available and the albumin preparations are inadequately standardized, the former was used in the subsequent experiments. The concentration of bovine serum on freezing was increased to 100%, but since it is toxic in high concentrations, after thawing the cells were washed and resuspended in Eagle's medium containing 20% of this serum. These results were similar to those of the positive control, in which autologous serum was used (Table 3).

On the basis of these results the lymphocytes of several donors were typed before and after freezing. They were frozen with 12.5% DMSO in both autologous and bovine serum, and the cell concentration was increased to $13 \cdot 10^6$ cells/ml, because of the size of the shakers and the number of lymphocytes required. Titration was carried out with 44 alloantisera against antigens of the HLA system. The results showed that, despite the general nonspecific increase in intensity of the reactions, caused by the higher proportion of dying cells, antigens determining the strongly positive reactions as before were detected clearly after freezing in both autologous and bovine serum. Simultaneous tests of the same cells with antilymphocytic globulin in dilutions of up to 1:256 also virtually coincided before and after freezing.

It can accordingly be concluded that the optimal medium for freezing human lymphocytes is autologous serum with 12.5% DMSO. In its absence, AB serum or bovine serum can conveniently be used. The latter (in concentrations over 20%) is best added to the cells only immediately before freezing, as is the case with DMSO also. It is very important to ensure that the temperature falls at the rate specified above. Thawing is best carried out as quickly as possible, but the cells must not be overheated. The cells can be resuspended after thawing in 20% bovine serum made up in any nutrient medium for tissue culture.

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