Table 1. Phenotype frequencies of HLA-BW51 in Japanese patients with Graves' disease or diabetes mellitus

	Control	Graves	JOD	MOD
(+)	4	9	1	3
(−)	75	49	39	26
χ^2		4.265	0.456	0.935
p		<0.05	NS	NS
Corrected p		NS		

Table 2. Phenotype frequencies of HLA-BW52 in Japanese patients with Graves' disease or diabetes mellitus

	Control	Graves	JOD	MOD
(+)	22	1	1	8
(−)	57	57	39	21
χ^2		16.213	10.853	0.023
p		<0.001	<0.001	NS
Corrected p		<0.002	<0.002	
			$\chi^2=9.241$	

Results. Table 1 shows the phenotype frequency of HLA-BW51 in Japanese patients with Graves' disease or diabetes mellitus and the controls. The frequencies of this antigen were 15.5% and 5.1% in the patients with Graves' disease and the healthy controls, respectively. The frequency of BW51 in the patients with Graves' disease showed a tendency to increase, but this was not statistically significant following correction for the number of antigens (corrected $p < 0.10$). There was no difference in the frequencies of this antigen among JOD patients, MOD patients and the controls.

Table 2 demonstrates the frequencies of HLA-BW52 in the patients and the controls. The frequencies of BW52 were 1.7% and 27.8% in the patients with Graves' disease and the healthy controls, respectively. Thus the frequency of this antigen in the Japanese patients with Graves' disease was significantly lower than that in the controls (corrected $p < 0.002$). The frequency of BW52 was significantly decreased also in JOD patients as compared with the controls (corrected $p < 0.002$) or with MOD patients (corrected $p < 0.02$). There was, however, no significant difference between MOD patients and the controls.

Discussion. To our knowledge, no report has been presented so far concerning the frequencies of HLA-BW51 and BW52 in Japanese patients with Graves' disease and JOD. The present study demonstrated that a decreased frequency of HLA-BW52 is a common characteristic shared by Japanese patients with Graves' disease and JOD. This finding seems to be noteworthy, since there is ample evidence that, in both conditions, abnormalities in immune responses play an important role in the development of the diseases. Though it is well-known that among Causasians an increased frequency of B8 is a common characteristic of the HLA phenotype in patients with Graves' disease[5] and JOD[6], there is no such common antigen(s) in the case of a decreased frequency. It appears noteworthy that among Japanese there is no common characteristic in the 2 endocrine diseases mentioned above as far as an increased frequency is concerned[1,2], but a decrease in the frequency of BW52 is found in both Graves' disease and JOD. The clinical implication of this finding is still to be elucidated, since no report has been presented concerning the relation between immune capacities and BW52 in the Japanese population.

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Preparative separation of nucleated cells from human bone marrow[1]

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Summary. A comparison has been made between the separation of nucleated cells from human bone marrow aspirates by high mol. wt polymers and the buffy coat techniques.

Cell separation is a critical problem for the study of hormone response on bone marrow cells when many different precursor types are present. One may expect in bone marrow aspirates a marked dilution of the nucleated cells by red cells. To reduce erythrocyte contamination aqueous solutions of high mol. wt polymers such as dextran, ficoll, and albumin have been used for a more effective nucleated cell separation[2,3]. A comparison has been made between the separation of nucleated bone marrow cells by high mol. wt polymers technique, and the collection of nucleated cells by centrifugation and separation of the buffy coat. While the buffy coat method is not as effective in the removal of red cells, it does provide a better yield of viable nucleated cells.

Materials and methods. Human bone marrow aspirates (3 ml), diluted with sterile phosphate-buffered saline (PBS) (10 ml) containing 167 U/ml of beef heparin were carefully passed through 19 and 23 gauge needles. The cell suspension after filtration through gauze represents the whole bone marrow (WBM). A suspension of WBM (400×10^6 cells/ml) was mixed with Dextran 75 in 0.9% saline (Abbott Lab.) in a ratio of 5:1 and allowed to settle on ice for 30 min. The suspended cells were removed and washed twice with PBS. A WBM suspension or a post-Dextran cell-PBS suspension (400×10^6 cells/ml) were layered over Ficoll-Paque solution (Pharmacia) in a ratio of 5:4 and spun at $400\times g$ at 12 °C for 40 min. The cells at the interface were harvested and washed twice with PBS.

When the buffy coat method was used, WBM cells in PBS were spun at $600\times g$ at 10 °C for 10 min. The cell pellet was resuspended in PBS and pipetted into a Kolmer buffy coat tube and spun at $1800\times g$ for 10 min at 10 °C. The buffy

Table 1. Ratio of erythrocytes to nucleated cells in human bone marrow aspirates after separation by aqueous polymers and by the buffy coat method

Method	Erythrocyte/nucleated cells ratio	Nucleated cells enrichment	Viability (%)
Whole bone marrow	83.70±21.4* (10)**	1.0	96-98
Dextran	17.00± 8.3 (4)	4.7	87-93
Dextran plus Ficoll	0.32± 0.11 (4)	63.7	88-93
Ficoll	0.42 (2)	59.2	88-93
Buffy coat	2.70± 0.41 (4)	22.7	96-98

* Mean ± SD. ** Number of experiments.

Table 2. Recovery of each kind of cell precursor in human bone marrow aspirates after separation with polymers and the buffy coat technique

Cell type	Recovery (%) after			
	Dextran (4)*	Ficoll (2)	Dextran + Ficoll (4)	Buffy coat (4)
Erythroid	23-35	12-14	3- 9	29-39
Myeloid	54-98	19-29	5-13	50-79
Lymphoid	20-49	60-66	12-22	36-68

* Number of experiments.

coat cells in the narrow neck of the tube were carefully collected with a Pasteur pipette and resuspended in PBS. Aliquots were taken of the cell-PBS suspension after each procedure for cell count, viability determination[4] and Wright's staining for identification of cell type.

Results. Table 1 shows the ratio of erythrocytes to nucleated cells after separation under the various conditions used. With Dextran 70-90% of the erythrocytes were excluded, with a 5-fold enrichment of nucleated cells. Ficoll treatment eliminated more than 98% of the erythrocytes with a 59-fold enrichment of nucleated cells. The sequential separation in Dextran and Ficoll improved neither the ratio nor the enrichment. However, either Dextran, Ficoll or the sequential separation decreased cell viability. The buffy coat method removed 95-99% of the erythrocytes, which represents a 23-fold enrichment in nucleated cells, with no changes in cell viability. A buffy coat separation followed by Ficoll, slightly improved the ratio to that obtained after the buffy coat procedure; however, the recovery of cells was poor (42%).

The recovery of each kind of nucleated cell after the various separations is shown in table 2. The yield of erythroid cells was higher with the buffy coat technique (30-40%) than with Dextran (23-35%) or with Ficoll (3-11%). The yield of myeloid cells was high after Dextran or the buffy coat as compared to that obtained after Ficoll or Dextran plus Ficoll procedures. The recovery of lymphoid cells was better with Ficoll than with the other methods.

Discussion. Of the 4 methods studied for the removal of contaminating erythrocytes from human bone marrow aspirates, the Ficoll and the Dextran plus Ficoll were very effective. However, they decreased cell viability, as judged by the dye exclusion technique. The buffy coat method, is not as effective for the removal of erythrocytes, but it is the best technique for a preparative separation of nucleated cells with good viability. Also, with this mild technique, the possible damage to cells caused by polymers may be avoided[2,5]. The choice of these methods depends on the cell type to be studied, since the yield of different marrow cell will vary with each method.

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Influence of ethanol on thyroxine accumulation in the hypothalamus, pituitary gland and cerebrospinal fluid in the newborn rabbit

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Summary. The effect of ethanol on thyroxine (T_4) accumulation in the hypothalamus (H), pituitary gland (P) and cerebrospinal fluid (CSF) has been investigated in 1-15-day-old rabbits. It has been found that H or CSF serum ratios decreased with age by about 2 in the course of 13 postnatal days. Stable T_4 resulted in an increase of ^{125}I-T_4 in H, P and CSF. Ethanol per se caused an increase in transfer and accumulation of radiothyroxine or made the changes after loading animals with carrier T_4 more pronounced.

The present experiments pertain to the blood-brain barrier transfer of labelled L-thyroxine-^{125}I during the first 2 weeks of life, i.e. in the period of a rapid growth and differentiation of the rabbit brain[1,2] and a progressive decrease in the transfer of the thyroid hormone across the blood-brain barrier[3]. They were aimed at finding a method of pharmacological alteration of the blood-brain barrier permeability for thyroxine as a necessary step for further investigations. Ethanol has been chosen as a substance acting on the barrier, as it was revealed in some preliminary observations that the thyroxine accumulation in the hypothalamus varies depending on whether given in ethanol or in physiological saline.

Materials and methods. Animals. Newborn rabbits of the Polish White breed kept with the does under normal farm conditions were used. The observations were carried out in series, each including 3 litters of 1-2-, 7- and 14-15-day-old rabbits. The animals received an injection of radioactive material, 3-5 μCi per 50 g b.wt in a volume of 0.6 ml into a jugular vein. The rabbits were killed by decapitation in a light chloroform-ether anaesthesia 30-60 min post-injection, and the whole pituitaries (P) and hypothalamic regions (H) (from optic chiasm to the anterior border of the pons) were removed. Blood samples were drawn from the heart. Cerebrospinal fluid (CSF) was collected with the aid of a 50-μl constriction pipette.