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.

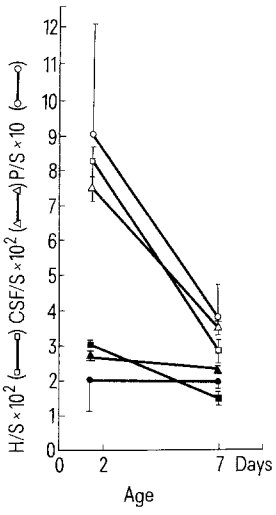

Fig. 1. An increase in the H/S, CSF/S and P/S ratios in newborn rabbits after i.v. administration of ^{125}I-T_4 in ethanol (white squares, triangles and circles) in comparison with controls (black symbols). Both the control and ethanol groups were receiving the labelled hormone in carrier (T_4, 10 µg/50 g b.wt). Note the diminished transfer of thyroxine with age independently to treatment.

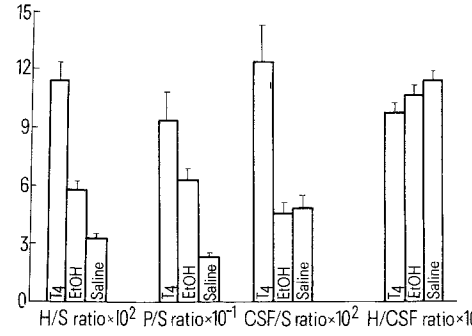

Fig. 2. Changes in the ^{125}I-T_4 tissue/serum ratios in 1-day-old rabbits after administration of the labelled hormone with thyroxine carrier (T_4, 10 µg/50 g b.wt), ethanol or saline. The animals were killed 60 min after i.v. injection of radiothyroxine (mean values ± SE; n = 4–5).

Radioactive material. Sodium L-thyroxine-^{125}I (sp. act. 25–35 mCi/mg) was purified by means of a gel filtration on Sephadex G-25 and its purity was checked by ascending chromatography in 2 solvent systems: n-butanol:dioxane 4:1, v/v satured with 2 M ammonia and n-butanol: glacial acetic: water (75:10:15, by vol.) according to Gross[4]. The accumulated radioactivity in tissues cleaned from any blood vessels and rinsed with physiological saline was measured and expressed as the tissue/serum (S) ratio.

Treatment. Powdered L-thyroxine, T_4 (Reneal, Budapest, Hungary) used as a non-radioactive carrier was given at the dose of 10 µg/50 g b.wt. Pure ethanol diluted to concentration 25% with saline was slowly injected i.v. with or without carrier T_4, in a volume of 0.6 ml/50 g b.wt.

Results. There was a much more rapid and intense accumulation of labelled thyroxine (T_4 carrier less than 0.14 µg/50 g b.wt) in 1-day than in 7–14-day-old animals (4 animals in each age group). The $H/S \times 10^2$ or $CSF/S \times 10^2$ ratios decreased with age by about 2 in the course of 13 postnatal days, i.e. from 4.13 ± 0.159 and 2.37 ± 0.281 to 2.2 ± 0.068 ($p < 0.001$) and 1.36 ± 0.072 ($p < 0.02$) respectively (means ± SE).

Injections of thyroxine in ethanol (EtOH · T_4 group) resulted in an increased T_4 accumulation in the hypothalamus (H), CSF and pituitary gland (P) (figure 1). In animals less than 1 day old (EtOH · T_4 group) the H/S, CSF/S and P/S ratios were about 3 times greater ($p < 0.001$) than in corresponding controls. At the age of 7 days, the thyroxine transfer was diminished independently to treatment. In spite of this decrease with age, H/S ratio was still significantly ($p < 0.02$) higher in animals receiving stable thyroxine in ethanol (figure 1).

In the series of experiments in which ethanol was given independently to thyroxine and to physiological saline (figure 2), ethanol per se caused a significant increase in H/S ($p < 0.01$) and P/S ($p < 0.001$) ratios in comparison with controls. The direction of changes was identical to which resulted after loading animals with thyroxine alone.

Discussion. With the exception of the previous paper[3], there is a lack of information on the blood-brain barrier permeability for thyroxine in newborns, and on the transfer of the endogenous thyroid hormones to the developing brain. Ford and Rhines[5] studied the effect of age on the pattern of labelled triiodothyronine accumulation in 2–14-week-old rats and found a progressive increase in the triiodothyronine accumulation in some parts of the brain from 2 till the age of 8 weeks. According to Bleecker, Ford and Rhines[6], this progressive increase in triiodothyronine accumulation mirrors the maturational phase that is an elevation of the amount of intracellular protein and protein-binding sites for thyroid hormone. These experiments are not comparable to ours, not only because of species differences and a different hormone concerned but basically because they started at age in which the postnatal process of brain maturation of the brain growth spurt were near to their end, whereas our studies began at least in its middle phase of perinatal development[7].

The present results of experiments obtained in control animals confirmed the previous observations[3] and showed that the younger the rabbit is the more the thyroid hormone enters the brain tissue. Injection of carrier thyroxine increased the transfer and its accumulation in the brain. As the free or unbound fraction of the plasma thyroid hormone is an active form of the hormone, and its level can be easily elevated by displacement from the protein carrier by loading blood with nonradioactive thyroxine[8], the obtained result is easily explained by the fact of increased free hormone fraction after loading with thyroxine and by enhanced passage to the peripheral tissue. On the other hand, more rapid accumulation of radiothyroxine in animals not loaded with carrier but receiving only ethanol might be considered as resulting from increased permeability of the blood-brain barrier for thyroxine due to ethanol action on the barrier.

Similarly, lower transfer and accumulation of thyroxine with age could be interpreted as a result of decreasing blood-brain barrier permeability for thyroxine and of higher resistance of the barrier to ethanol action. An increase in the plasma protein-binding capacity lowering the free hormone level, already observed in some species[9], could not be completely eliminated as an additional modifying factor in older rabbits.

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