evidently the first time that these data have been obtained. They indicate that the lymphocyte contains a natural oscillator (oscillators) which, in particular, control the circadian rhythm of activity of the E receptor. According to existing views, circadian variations in lymphocyte subpopulations contained in the blood (including cells forming E rosettes) are connected with circadian changes in the plasma cortisol level and with the dynamics of release of cells from the lymphoid tissues into the circulation [4, 10, 12]. In the light of our own data obtained with cells in vitro, rhythmic fluctuations in the subpopulation composition of the blood lymphocytes may depend on circadian changes in the cells themselves and, in particular, their receptors used for identifying subpopulations.

Circa-annual rhythms have a significant influence on the character of the circadian rhythms of E rosette-formation studied in this investigation: in summer circadian fluctuations of rosette formation were significantly reduced in amplitude. All the investigations described in this paper were therefore carried out in February and November.

LITERATURE CITED

1. S. S. Laguchev and A. I. Pivovarova, Dokl. Akad. Nauk SSSR, 179, 493 (1968).
2. V. P. Lozovoi, S. M. Shergin, and A. A. Povazhenko, Byull. Éksp. Biol. Med., No. 7, 872 (1976).
3. T. Abo and K. Kumagai, Clin. Exp. Immunol., 33, 441 (1978).
4. T. Abo, T. Kawate, K. Itoh, and K. Kumagai, J. Immunol., 126, 1360 (1981).
5. G. Casale and P. de Nicola, Arch. Gerontol. Geriat., 3, 267 (1984).
6. G. Fernandes, F. Garandente, E. Halberg, et al., J. Immunol., 123, 622 (1979).
7. M. S. Kaplan, V. S. Byers, A. S. Levin, et al., J. Allergy, 58, 180 (1976).
8. F. Levi, C. Canon, J. P. Blum, et al., Lancet, 2, 462 (1983).
9. F. Levi, C. Canon, J. P. Blum, et al., J. Immunol., 134, 217 (1985).
10. T. Miyawaki, K. Taga, T. Nagaoki, et al., Clin. Exp. Immunol., 55, 618 (1984).
11. M. S. Moore-Ede, Fed. Proc., 42, 2802 (1983).
12. A. W. S. Ritchie, I. Oswald, H. S. Micklem, et al., Br. Med. J., 286, 1173 (1983).
13. C. F. Shield, P. Marleu, A. Smith, et al., J. Immunol. Meth., 62, 343 (1983).
14. R. Smith, R. Kerman, E. Ezdinli, and S. Stefani, J. Immunol. Meth., 8, 175 (1975).
15. R. M. Williams, L. J. Kruas, D. P. Dubey, et al., Chronobiologia, 6, 162 (1980).

FLOATING CULTURES OBTAINED FROM HUMAN FETAL THYROID GLAND

V. N. Blyumkin, L. A. Kirsanova,
N. N. Skaletskii, R. P. Babikova,
and Yu. V. Kiprenskii

UDC 615.012.41:612.379.7

KEY WORDS: thyroid glands; cell culture; transplantation.

Experience with clinical transplantation of cultures of endocrine cells has accumulated in recent years. It can be regarded as one method of replacement therapy in the treatment of hormone disturbances. There is evidence that cultures can be obtained from human and animal thyroid glands [3, 6-10, 12]. Investigations [8] which showed that preliminary culture of thyroid gland tissue can considerably prolong its life in an allogeneic or xenogeneic host on subsequent transplantation are particularly interesting. According to Lafferty's hypothesis, this is connected with elimination of what are called "passenger leukocytes" — costimulator cells of the immune response. The problem of culture of thyroid gland cells is important in connection with their potential use for clinical transplantation into hypothyroid patients not responding to ordinary medication. Accordingly it was decided to study the possibility

Tissue Culture Laboratory, Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 600-602, November, 1988. Original article submitted December 18, 1987.

0007-4888/88/0011-1616$12.50

of obtaining cultures of this kind. The writers previously developed a technique for obtaining organotypical floating cultures of human and animal fetal pancreas [1]. Their structure and some floating cultures possess considerable insulin-producing activity [1, 4]. Long-term function of cultures in rats with experimental diabetes has been shown to be possible [2, 5]. At present floating cultures prepared from the thyroid gland are used for allografting and xenografting into patients with insulin-dependent diabetes [11].

The technique developed for obtaining organotypical protein cultures of the human and animal fetal pancreas was tested, with certain modifications depending on the histological features of the thyroid gland, to study the possibility of using it on the human fetal thyroid gland.

EXPERIMENTAL METHOD

Thyroid glands from cadavers of human fetuses at 15-20 weeks of intrauterine development were used for obtaining floating cultures. The thyroid gland was removed under sterile conditions, thoroughly washed in Hanks' solution with antibiotics (penicillin 500 U/ml, streptomycin 500 µg/ml, or kanamycin 500 U/ml), and freed from capsule and large connective-tissue membranes with blood vessels. The gland was cut into fragments measuring 2-3 mm, washed to remove blood, and treated with a 0.15% solution of collalytin in Hanks' solution. The material was exposed to collalytin for about 5 min at room temperature. The thyroid gland fragments were then washed several times with medium 199 and carefully cut up into small pieces with ophthalamic scissors in order to obtain microfragments measuring about 1 mm. The minced tissue was transferred to sterile flasks of varied capacity, and covered with appropriate amounts of medium 199 with 15% bovine serum and antibiotics. The material was incubated at 37°C. The cultures were harvested with a Pasteur pipet for histological investigation at different times of growth, washed in medium 199, and fixed with Bouin's mixture. After dehydration in alcohols of increasing concentration the material was embedded in paraffin wax. Sections 5-7 µ thick were stained with hematoxylin and eosin and by Van Gieson's method.

EXPERIMENTAL RESULTS

During culture of thyroid gland microfragments obtained by the method described above, some fragments settled on the surface of the glass and adhered to it, but the overwhelming majority of microfragments remained freely floating in the growth medium. Thus the cultures separated into settling and floating fractions. During the first 2-3 days the surface of the microfragments was overgrown with fibroblasts and the microfragments themselves became regularly spherical or ovoid in shape. During its survival in culture the fibroblast membrane developed changes: from a monolayer in the early periods of culture (7-10 days) it was converted into a stratified structure at later stages (after 21-40 days). The parenchyma of the floating cultures was composed of epithelial cells (thyrocytes), forming follicles and compact concentrations, which evidently corresponded to interfollicular islets of the original material — fetal thyroid gland. Thyrocytes lining the cavity of the follicles in most cases were cubical or prismatic in shape, a characteristic feature of functioning follicles. The cavity of these follicles was filled with eosinophilic colloid. The colloid of some follicles contained cell debris. During culture individual follicles underwent lysis and only their "ghosts" remained. The stroma of the floating cultures was formed by bundles of relatively unchanged (delicately fuchsinophilic) and digested (picrinophilic) collagen.

To determine the hormone-producing activity of the thyrocytes of the cultures thus obtained samples of culture fluid were taken and their content of triiodothyronine and thyroxine was determined by radioimmunoassay. Hormones characteristic of the thyroid gland were discovered throughout the period of culture (40 days), during repeated and complete changes of the medium.

The method described above can thus be used to obtain organotypical floating cultures of human fetal thyroid gland. The cultures obtained preserved, in its general features, the structural organization of the original material and possess hormone-producing activity. The first trials of the use of these cultures in the clinic of the writers' Institute have demonstrated that they can be used for the treatment of patients with manifestations of hypothyroidism.

LITERATURE CITED

1. V. N. Blyumkin, N. N. Skaletskii, V. L. Popov, et al., Byull. Éksp. Biol. Med., No. 5, 89 (1983).

2. V. N. Blyumkin, N. N. Skaletskii, N. I. Kauricheva, et al., Byull. Éksp. Biol. Med., No. 6, 764 (1984).
3. I. S. Turchin, "The study of the structure and function of endocrine gland cells by the tissue culture method," Dissertation for the degree of Doctor of Medical Sciences, Kiev (1975).
4. V. I. Shumakov, V. N. Blyumkin, R. A. Babikova, et al., Byull. Éksp. Biol. Med., No. 8, 202 (1979).
5. V. I. Shumakov, B. I. Shal'nev, V. N. Blyumkin, et al., Byull. Éksp. Biol. Med., No. 1, 49 (1980).
6. E. Bell, H. Moore, C. Mitchie, et al., J. Exp. Zool., 232, No. 2, 277 (1984).
7. J. E. Errick, K. Ing, M. Eggo, et al., In Vitro, 22, No. 1, 28 (1986).
8. K. J. Lafferty, A. Bootes, G. Dart, et al., Transplantation, 22, No. 2, 138 (1976).
9. C. Mothersill, C. Seymour, M. J. Moriarty, et al., Acta Endocrinol. (Copenhagen), 108, No. 2, 192 (1985).
10. P. Pic, L. Pemy, A.-M. Athouel-Haon, et al., Cell Tissue Res., 237, No. 3, 499 (1984).
11. V. I. Shumakov, V. N. Blyumkin (V. N. Bljumkin), S. N. Ignatenko, et al., Transplant. Proc., 19, No. 1, 2372 (1987).
12. C. Tacchetti, C. Zurzolo, A. Monticelli, et al., J. Cell. Physiol., 126, No. 1, 93 (1986).

MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF THE FEMALE REPRODUCTIVE SYSTEM IN MICE OF THE MUTANT B10-hr^{rhY} LINE

E. L. Ignat'eva, A. M. Malashenko, and Z. K. Blandova

UDC 612.663.06:612.6.05/.08

KEY WORDS: mutation; hairless; sterility; ovary; homozygotes; transplantation.

A spontaneous autosomal recessive mutation causing loss of hair has been discovered at the Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR. The mutation is allelic with the hairless (hr) mutation in chromosome 14, but differs in its effect from known alleles of that locus. Phenotypically the mutants are similar to the rhino mutation, and the gene is designated by the symbol hr^{rhY} (hairless rhino—Yurlovo). The mutants are characterized by defects of the hair cover and of skin structure, by a defect of the immune system, and by sterility of the females [1]. The mutation has now been maintained on a genetic basis in line C57BL/10SnY (abbreviated to B10), by crossing heterozygous females with male hr^{rhY}/hr^{rhY} homozygotes. The heterozygous females have normal fertility, but ability of the males to reproduce is depressed, and by the age of 4-6 months they lose their fertility.

The aim of this investigation was to study the causes of sterility in females, for this is a characteristic that is unknown for other alleles of the hr gene which have been described, and it was also aimed to develop an effective method of breeding these mutants.

EXPERIMENTAL METHOD

Mice of inbred line B10 and mutant line B10-hr^{rhY} were used and bred under conventional conditions in the Department of Genetics, Research Laboratory of Experimental Biological Models. The animals were kept in T2 cages (VELAZ) and were fed on the granulated combined feed PK-120-3.

For cytological investigation of the estrous cycle in the mutants and mice of the isogenic line aged 2-6 months, morning vaginal smears were obtained daily for 2 weeks during the summer. For histological processing the ovaries of females whose vaginal smears had previously

Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 602-603, November, 1988. Original article submitted October 23, 1987.

 0007-4888/88/0011-1618$12.50