Effects of antithymocyte (ATS) and normal pig serum (NPS) on the estrous cycle in rats

Day of treatment	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Average score/day
	Vaginal smear score															
Rat No. 1	10	1	1	1	1	5	5	1	1	1	1	1	1	1		2.14
2	1	1	1	1	1	1	1	1	1	3	1	8	1	1		1.64
3	5	1	1	7	4	1	1	4	1	1	1	1	1	10		2.79
4	1	1	1	1	10	1	1	1	9	10	1	1	8	8		3.86
5	1	1	1	1	1	1	1	1	1	1	1	1	8	1		1.5
6	1	4	8	10	7	1	1	1	4	1	1	1	4	1		3.21
														Mean		2.52
														S. E.		0.38
														95% confidence interval		1.55–3.49
NPS																
Rat No. 1	1	2	8	10	3	1	1	7	10	8	10	1	1	4		4.79
2	5	10	3	1	1	7	10	1	1	7	8	8	1	7		5.0
3	1	2	7	10	3	1	1	7	9	1	1	8	7	1		4.21
4	1	1	5	10	3	1	1	2	7	8	10	1	8	7		4.64
5	7	10	3	1	1	7	10	3	1	1	5	10	2	1		4.43
														Mean		4.61
														S. E.		0.14
														95% confidence interval		4.23–4.99

* Smears taken on the first day of treatment were not evaluated. Different vaginal smear patterns were scored arbitrarily as follows: leucocytes – 1, leucocytes with nucleated epithelial cells – 2, leucocytes with cornified cells – 3, leucocytes, nucleated and cornified epithelial cells – 4, nucleated epithelial cells – 5 (score 6 was not used), nucleated epithelial cells with admixed cornified cells – 7, nucleated and cornified cells in equal proportions – 8, cornified cells with admixed nucleated epithelial cells – 9, cornified cells – 10.

while that of the experimental ATS-treated animals displayed a distinct tendency toward a diestrous pattern during treatment and a rapid and permanent recovery after terminating the treatment. The ovaries of the 3 experimental animals killed at the end of the ATS-influenced period did not differ histologically from those in the untreated animals; they contained numerous follicles of different ages and corpora lutea. The thymus and spleen in these animals revealed only an increased vascularization. The ovarian, thymic and splenic morphology in the experimental and control animals killed 2 months after the ATS or NPS treatments respectively was indistinguishable from that in the untreated rats.

We were able to suppress ovulation with a persistence of corpora lutea and a tendency toward a permanent diestrous state by influencing the immune system of adult rats with the administration of ATS in sexually mature animals. The NPS was ineffective; no difference could be found between the NPS-treated controls and untreated animals. Future research must answer the question of whether the immune system actually does effect the ovarian function directly by influencing the ovarian structures, or only indirectly by influencing the hypothalamus-pituitary system. We consider the former possibility more probable[6,7].

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Effects of TSH and cyclic AMP on the human thyroid cells cultured in a chemically defined medium

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Summary. In a serum-free, chemically defined medium human thyroid cells elongated remarkably and resembled fibroblastic cells. They retained the cyclic AMP response to TSH and the supplement of medium with TSH or dibutyryl cyclic AMP permitted the preservation of epithelial nature by the cells. Cyclic AMP of the cells of epithelial nature was higher than those of fibroblastic appearance.

Tissue culture is an excellent tool for studying the mechanism of hormonal actions[2,3]. Most cells in culture, however, require serum to survive and grow[4]. Serum is considered to provide necessary hormones, some as yet unidentified, which may act on target cells by themselves or as 'permissive factors' of other hormones[5,6]. This implies that the use of serum in tissue culture experiments on hormonal action complicates the interpretation of effects. If the cells are able to survive with their specific characters in a chemically defined medium, the serum-free system will pose a partial solution to this problem. It probably helps to characterize more precisely the mechanism of action of hormones and other serum factors[4,5]. This paper presents a trial to maintain human thyroid cells in a chemically defined medium with or without TSH.

Materials and methods. Human thyroid tissue was obtained at surgery from patients with Graves' disease who were euthyroid after the treatment with thiourea for 3–4 months. The thyroid cells were cultured in Ham's F12 medium with 10% calf serum at the density of 2×10^6 cells per 60 mm dish as reported previously[7]. After 24 h of incubation cells were washed 3 times with 5 ml of Ham's F12 medium (serum-free), and serum-free culture using Ham's F12 medium was started. Every 2 days the medium was replaced by new one with or without hormones or cyclic nucleotides[7]. Cell number was determined by staining and counting the nuclei by Absher's method[8]. Cyclic AMP was measured as described before[7].

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Results. In Ham's F12 medium the human thyroid cells remained attached to the surface of culture dish. The cell number was constant for at least a week, and was not changed by supplementing the medium with TSH (up to 100 mU/ml). At the beginning of serum-free culture, the cells showed round or polygonal shape with scanty cytoplasm. The cells gradually elongated and flattened. The elongation reached its maximum after 2–3 days and the cells displayed a tendensy to align in parallel as shown in figure 1. These cells, however, preserved the sensitivity to TSH(>1 mU/ml) for at least a week, and elevated cyclic AMP level from 7.2 ± 0.6 pmoles/10^6 cells to 71.2 ± 4.3 pmoles/10^6 cells after 20 min of incubation at 37 °C with 100 mU/ml of TSH (Armour, Thytropal).

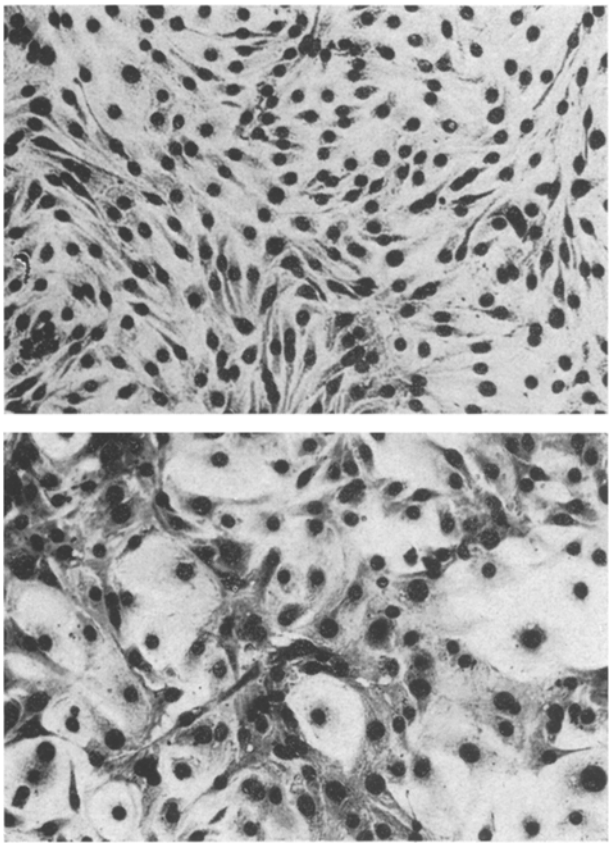

Fig. 1. Morphological appearance of thyroid cells maintained in Ham's F12 medium. Cells were maintained for 4 days in Ham's F12 medium with (lower) or without (upper) 100 mU/ml of TSH. The photographs were taken after cells were stained with May-Giemsa. × 100.

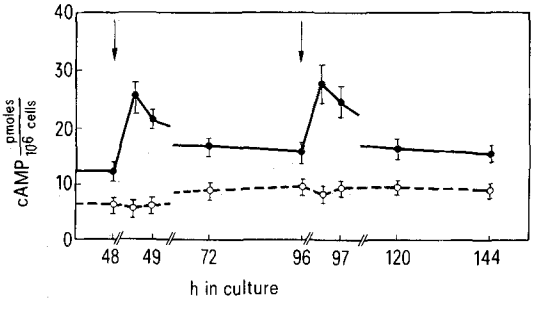

Fig. 2. Cyclic AMP levels of thyroid cells maintained with or without TSH. Cells were maintained in Ham's F12 medium with (closed circles) or without (open circles) 10 mU/ml of TSH. Medium was replaced every 2 days (arrows) by a fresh medium with or without TSH. After various length of serum-free culture (abscissa) cyclic AMP level was measured as described before[7]. Each point is the mean ± S. E. M. triplicates.

When the thyroid cells were cultured in Ham's F12 medium supplemented with TSH(>1 mU/ml), the cell elongation was not recognized. As shown in figure 1, they preserved the variability in both size and shape characteristic of differentiated epithelial cells in primary culture. The suppression of cell elongation by TSH seemed reversible since the removal of TSH permitted the cells to elongate. TSH had no effect on the fully elongated cells, while it prevented the process of cell elongation when added within 24 h after the beginning of TSH-free culture.

The cyclic AMP level of cells in TSH-free medium was 8.8 ± 0.6 pmoles/10^6 cells (4-day-old culture) and the medium change brought very little fluctuation in the basal level (figure 2, open circle). When medium was replaced by fresh one with 10 mU/ml of TSH, cyclic AMP level was elevated within 5 min. The level reached its maximum by 30 min, then followed by a gradual decrease (figure 2, closed circle). The lowest levels just before the next medium change (16.4 ± 0.7 pmoles/10^6 cells, 4-day-old culture) were still higher than those in control culture (figure 2, open circles), indicating that the cells of epithelial nature were in higher cyclic AMP levels throughout the culture than those with elongated cytoplasm. ACTH(100 mU/ml), HCG(20 U/ml) and bovine serum albumin(5 mg/ml) had no effect on the cyclic AMP level as well as the cell morphology. N^6,2′-O-dibutyryl cyclic AMP mimicked the TSH action on the cell morphology at the concentration of 0.01, 0.1 and 1 mM, while N^6,2′-dibutyryl cyclic GMP did not at the same concentration.

Discussion. In Ham's F12 medium human thyroid cells preserved the sensitivity to TSH, while their epithelial nature was lost as a remarkable cell elongation. TSH prevented the morphological change probably by elevating the cyclic AMP level. This is compatible with the previous reports and adds further support to the theory that the formation of tissue specific structure by thyroid cell is regulated by TSH-cyclic AMP system[2,3].

The mechanism of cell elongation and its inhibition by cyclic AMP is not known. The participation of microtubules as reported recently[9,10] seemed unlikely in thyroid cell elongation, since colchicin did not inhibit the elongation in our preliminary experiments. Some kinds of cell-to-cell interaction seemed to have an intimate relation to the morphological change because the elongation was seen only when the thyroid cells were seeded at a proper cell density. However, although involvement of cyclic AMP and membrane properties was postulated to explain such density dependent nature[11,12], exact mechanism by which cells change their morphology depending on their population remains to be studied.

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