ganic phosphate specific activities to that of the plasma (table 2). The exception to this were the striated muscles and brain. Both showed a low specific activity which indicates slower turnover. Parathyroid hormone significantly reduced the specific activity of phosphate in the plasma and most organs, which is consistent with the hypothesis of a release of low specific activity phosphate from the striated muscles. A decline in plasma specific activity would reduce the organ specific activity as the turnover of inorganic phosphate occurred between tissues and the plasma.

Discussion. The large phosphaturic effect of PTH in thyroparathyroidectomized rats indicated mobilization of phosphate from a source other than the ECF, but the phosphate content of most organs was unaffected by acute PTH administration[2]. The present study indicates that soft tissue phosphate release is restricted to inorganic phosphate from striated muscle and smaller amounts from both inorganic and esterified phosphate from liver and lung. Release of inorganic phosphate from muscle in the amounts of 1 μmole/g is sufficient to account for the large phosphaturic response to PTH observed earlier[2].

It should be emphasized that the mechanism of this release is unknown. There are numerous possible explanations including a direct effect of PTH, some unknown phosphate mobilizing hormone, or a response of the muscle cells to hypophosphatemia. The latter is unlikely since the hypophosphatemia following calcitonin administration does not alter muscle phosphate levels[4]. Also, if phosphate behaves as chloride does in the cell, the negatively charged cytoplasm would repel the phosphate and limit the quantity of free phosphate in the cytoplasm. Thus, most of the phosphate measured in our studies is probably stored in organelles. Phosphate is known to be actively accumulated by the mitochondria[8]. We suggest that the release of phosphate from muscle is from an organelle within the cell and is unlikely to represent simple diffusion out of the cytoplasm. We suggest that this large pool of slowly turned-over muscle phosphate may represent a physiological reserve which can be mobilized to prevent extreme hypophosphatemia.

8 N. E. Lofrumento, F. Zanotti and S. Papa, FEBS Lett. *48*, 188 (1974).

Ovarian function in adult rats treated with antithymocyte serum

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Summary. Intraperitoneal treatment of adult rats with antithymocyte serum for 2 weeks altered the ovarian function. During the treatment, anovulation with the persistence of corpora lutea and a tendency toward a permanent diestrous state was observed.

Recently, a role of thymus during the perinatal period with respect to the adult ovarian function has been suggested. The thymus may well have a basic role in the organization of the late adult hypothalamus-pituitary axis for sexual functions by programming the functions of the neuroendocrine system[1]. A neonatal thymectomy induced sterility in female mice[2] and could be prevented by the grafting of thymuses from newborn or adult mice within 14 days after birth[3]. Thymectomy after the age of 7 days was no longer associated with disturbed ovarian development[2]. An intraperitoneal injection of a thymic cell suspension from 22- to 30-day-old female rats into newborn rats prevented the adult anovulatory sterility induced by a single testosterone propionate administration at 5 days of age[4]. All these results demonstrate a possible participation of the immune system in the development of the ovarian function. This study was designed to determine the contingent changes in the ovarian function, estimated by vaginal smear changes, induced by the antithymocyte serum in adult rats.

Antithymocyte serum (ATS) was produced by immunizing pigs weighing 80–90 kg with thymus cells taken from 6-week-old outbred rats. The immunization procedure included 2 s.c. injections spaced 14 days apart, each containing 2×10^9 cells in complete Freund's adjuvant. 7 days after the second injection the animals were bled and the serum was collected, inactivated at 56 °C for 30 min and absorbed with one-half of its volume of rats erythrocytes. The cytotoxin, leukoagglutinin and hemagglutinin titers of the serum were 1:256, 1:2048 and 1:64 respectively. The immuno-suppressive effects of ATS were assessed by producing a prolonged semiallogenic graft survival in rats[5]. Normal pig serum (NPS) was processed in the same manner.

Adult, 9-week-old female Wistar rats of our laboratory colony, displaying regular estrous cycles for 14 days before the experiment, were used. In the experimental group, 6 animals were injected intraperitoneally with 1.5 ml of ATS daily for 15 days. 5 control animals were treated with equal amounts of NPS. Vaginal smears were taken daily from the second day to the last day of treatment from both experimental and control groups. These were evaluated by ascribing arbitrary numbers to different cytological patterns (table), and the average daily score was determined for each of the animals during the ATS or NPS administration, in order to evaluate the differences statistically. The average vaginal score in the ATS-treated group (2.52) was significantly lower ($p < 0.01$) than that in the NPS-treated group (4.61). By way of comparison, the average vaginal score in a group of untreated animals of the same age observed for 28 days was 4.71 (S.E. 0.29; 95% confidence interval 3.46–5.98).

3 experimental animals were killed immediately after terminating the ATS treatment; estrous cycles in the remaining experimental and all control animals were studied during the 2 following months and the rats were killed afterwards. The estrous cycle of the control NPS-treated rats remained normal during and after treatment,

1 W. Pierpaoli and H. O. Besedovsky, Clin. exp. Immun. *20*, 323 (1975).
2 Y. Nishizuka and T. Sakakura, Endocrinology *89*, 886 (1971).
3 T. Sakakura and Y. Nishizuka, Endocrinology *90*, 431 (1972).
4 F. A. Kincl, A. Oriol, A. Folch Pi and M. Maqueo, Proc. Soc. exp. Biol. N. Y. *120*, 252 (1965).
5 V. Hašková, L. Korčáková and T. Bednařík, Transplantation *16*, 325 (1973).

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].

6 A. Bukovský, J. Presl and Z. Krabec, Čs. Gynek. *41*, 281 (1976).
7 J. Presl and A. Bukovský, Čs. Gynek. *41*, 449 (1976).

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].

1 Present address: Dept. of Med., Albert Einstein College of Medicine, Bronx (New York 10461, USA).

2 P. R. Kerkof, J. P. Long and I. L. Chaikof, Endocrinology *74*, 170 (1964).
3 S. Lissitzky, G. Fayet, A. Giraud, B. Varrier and J. Forresani, Eur. J. Biochem. *24*, 88 (1971).
4 G. H. Sato, in: Biochemical Action of Hormones, vol. 3, p. 391. Ed. G. Litowack. Academic Press, New York–London 1975.
5 I. Hayashi and G. H. Sato, Nature *259*, 132 (1976).
6 D. K. Granner, Nature *259*, 572 (1976).
7 Y. Kaneko, Horm. Metab. Res. *8*, 202 (1976).
8 M. Absher, in: Tissue Culture Methods and Applications, p. 395. Ed. P. F. Kruse, Jr, and M. K. Patterson, Jr. Academic Press, New York–London 1973.