during non-hibernation. The animals were divided equally into 3 groups. A single subcutaneous injection of 0.5 mg of cadmium chloride was given to 2 groups of animals and the control group received 0.2 ml of amphibian saline. The animals of one treated group were killed after 7 days, simultaneously with the animals of other treated group after 3 days of cadmium injection and controls after 7 days of saline injection. One testis from each animal was fixed in Bouin's fluid for histological study while other testis was used for histochemical localization of Δ^5-3β-HSD. The measurement of Leydig cells and its nuclear area was performed on the slides stained with hematoxylin and eosin according to the method of Deb et al.[9]. For histochemical demonstration of Δ^5-3β-HSD fresh frozen sections were cut into 20 μm on a Cryostat. Δ^5-3β-HSD activity in the sections of testis was determined in a substrate medium (dehydro epiandrosterone) as described by Deane et al.[10]. Parallel sections incubated in a substrate-free medium served as controls. After 60 min incubation at 37 °C all the sections were fixed and mounted in glycerine jelly.

Histochemical reactions showed Δ^5-3β-HSD activity in both the tubular and Leydig cells of the control toad testis (figure 1). The area of the Leydig cells including their nuclei (table) and the activity of the enzyme Δ^5-3β-HSD (figure 2) in the testis of cadmium-treated toad sacrificed after 7 days, appeared to be increased significantly compared with that of controls and the treated animals that received cadmium 3 days before sacrifice. The seminiferous tubulus of the treated animals injected with cadmium 7 days before showed no activity of the enzyme. The area of the Leydig cells and the activity of Δ^5-3β-HSD in the testis of toad sacrificed 3 days after cadmium injection revealed no significant change as compared with that of controls.

Effect of cadmium on the Leydig cell and nuclear area

Group	Leydig cell area* (cm^2)	Leydig cell nuclear area * (cm^2)	No. of toads
Control	0.85 $\pm$ 0.04**	0.32 $\pm$ 0.01	5
3 days	0.88 $\pm$ 0.11	0.33 $\pm$ 0.03	5
7 days	1.26 $\pm$ 0.04	0.47 $\pm$ 0.01	5
p-value; control vs 3 days	NS	NS	
p-value; control vs 7 days	$<$0.001	$<$0.001	
p-value; 3 days vs 7 days	$<$0.02	$<$0.001	

* Camera Lucida, $\times$ 500. ** Mean $\pm$ SE. NS indicates statistically not significant.

The present study shows that the extension of period from 3 to 7 days, after cadmium injection in toad, results in Leydig cells hypertrophy and stimulation of Δ^5-3β-HSD activity in the testis. Presence of Δ^5-3β-HSD in both the tubular and Leydig cells in the toad testis has been reported previously[11,12]. Since the enzyme Δ^5-3β-HSD plays an important role in steroid hormone synthesis, the present observations indicate that testicular hormone synthesis is possibly increased in Leydig cells while decreased in tubular cells after cadmium injection in toad. Gunn et al.[7] have demonstrated Leydig cell proliferation and tumor in the testis of rat treated with cadmium. They have suggested that the tumors are capable of secreting sufficient estrogen. Leydig cells hypertrophy has also been noted in the cadmium-treated pigeon[8]. But the mechanism through which cadmium stimulates Leydig cell activity has not been elucidated. Reports of a high ICSH-activity of the anterior pituitary has been reported by Kar et al. in the cadmium-treated mice[3,13]. Recently, histological studies on the anterior pituitary reveals that LH secreting gonadotrophs are increased in the cadmium-treated toad[14]. The above evidence, therefore, suggests that cadmium chloride stimulates Leydig cells' activity, possibly by increasing gonadotropin synthesis.

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Phosphate mobilization from striated muscle following parathyroid hormone administration to thyroparathyroidectomized rats[1]

R. A. Meyer, Jr, and Martha H. Meyer

Department of Basic Science, Marquette University School of Dentistry, 604 North Sixteenth Street, Milwaukee, Wisconsin 53233 (USA), 17 August 1976

Summary. Parathyroid hormone administration to thyroparathyroidectomized rats resulted in a significant reduction in inorganic phosphate content of a variety of striated muscles. Most other organs were unaffected. Much of the extra urinary phosphate present after parathyroid hormone stimulation may be released from the muscles.

Introduction. We have proposed that the phosphaturic effect of parathyroid hormone (PTH) involves more than the renal action of the hormone which transfers phosphate from the extracellular fluid (ECF) into the urine. We have suggested that there is also significant release of phosphate from certain soft tissues, especially muscle, during the phosphaturia and that this muscle phosphate may be the source of most of the extra phosphate appearing in the urine[2]. This concept is supported by inferences made from urinary phosphate data, although negative and opposite urinary results have also been presented[2]. Our first data were limited to the testing of one muscle, and our inorganic phosphate assay gave somewhat higher values than those of Hansen et al.[3], suggesting some contamination with esterified phosphate. The purpose of this study was to examine other muscles and organs in the rat to more completely describe the origins of the PTH induced phosphaturia. In addition, the inorganic phosphate assay was improved to more accurately separate changes in inorganic from changes in esterified phosphate.

Table 1. Effect of PTH on phosphate levels

Sample	Experiment	n	Vehicle	PTH	Δ*	p**	Vehicle	PTH	Δ	p
			Inorganic phosphate				Acid soluble phosphate			
Striated muscle										
Diaphragm	1	19	14.6***	13.4	−1.2 ± 0.4	0.01	31.9	29.7	−2.2 ± 1.1	0.07
Esophagus	2	10	10.5	9.6	−1.0 ± 0.2	<0.01	22.2	20.7	−1.4 ± 0.7	0.07
Gastrocnemius	2,3	21	19.0	18.4	−0.6 ± 0.3	<0.1	45.6	45.6	0.1 ± 0.5	NS
Soleus	2,3	20	14.4	13.6	−0.9 ± 0.2	<0.001	32.6	30.8	−1.8 ± 0.5	<0.01
Other muscle										
Intestinal smooth	3	11	6.90	6.61	−0.29 ± 0.38	NS	15.0	15.0	0.0 ± 0.5	NS
Ventricle	1	19	11.7	11.2	−0.4 ± 0.4	NS	22.0	21.4	−0.6 ± 0.4	NS
Other organs										
Cerebral cortex	2,3	22	7.14	7.03	−0.11 ± 0.34	NS	14.5	14.3	−0.2 ± 0.4	NS
Liver	2,3	21	3.78	3.25	−0.54 ± 0.14	<0.001	18.4	17.3	−1.2 ± 0.4	<0.01
Lung	1	20	3.81	3.46	−0.36 ± 0.18	0.06	8.7	7.7	−1.0 ± 0.2	<0.001
			Plasma minerals							
Inorganic phosphate	1,2,3	42	3.51	2.33	−1.18 ± 0.04	<0.001				
Total calcium	1,2,3	42	1.61	2.81	1.20 ± 0.05	<0.001				

*$\overline{X} \pm$ S. E. for the difference between PTH and vehicle treated rats in each of n replicates. **Calculated by the t-test for paired observations[5]. ***μmole/g tissue wet weight except for plasma data which are μmole/ml.

Materials and methods. Our methods followed the tissue studies published earlier[2] except as noted. Male, SPD rats, from ARS/Sprague-Dawley, Madison, Wisconsin, arbitrarily paired, were thyroparathyroidectomized. After surgery, the animals were given distilled water ad libitum but no food. 12 h later, 100 μCi $^{32}PO_4$ were administered intravenously in 0.5 ml 0.9% NaCl. 5 min later a s.c. injection of either 50 U PTH (Wilson Laboratories, Chicago, TCA powder of bovine PTH, 179 U/mg) dissolved in 0.2 ml of 0.9% NaCl or a vehicle (V) injection of 0.2 ml 0.9% NaCl was given at random to each pair of rats.
4 h later, the rats were anesthetized and a blood sample was collected from the tail for plasma calcium and inorganic phosphate assay. Tissues were collected for inorganic and acid soluble phosphate assay with the following modifications. Tissue samples were frozen in liquid nitrogen. Each frozen sample was weighed and the tissue was homogenized with a motor-driven pestle at 0 °C in 2.5 ml 5% $HClO_4$ while still frozen. The inorganic ^{32}P level was measured[4], and specific activity was calculated.

Table 2. Effect of PTH on ^{32}P specific activity

Sample	n	Vehicle	PTH	Δ	p
		Inorganic phosphate SA			
Striated muscle					
Diaphragm	18	6.85*	5.67	−1.17 ± 0.28	< 0.001
Esophagus	11	7.22	5.20	−2.03 ± 0.31	< 0.001
Gastrocnemius	22	2.38	2.21	−0.13 ± 0.16	NS
Soleus	21	6.85	6.17	−0.68 ± 0.62	NS
Other muscle					
Intestinal smooth	11	18.1	15.8	−2.3 ± 0.7	0.01
Ventricle	19	30.1	25.8	−4.3 ± 0.9	< 0.001
Other organs					
Cerebral cortex	22	1.65	1.39	−0.26 ± 0.08	< 0.01
Liver	21	32.1	26.4	−5.8 ± 0.9	< 0.001
Lung	19	26.9	18.8	−8.1 ± 1.1	< 0.001
Plasma	41	28.2	19.4	−8.8 ± 0.7	< 0.001

*cpm × 10^{-3}/μmole P. The data are presented as in table 1.

Tissues were collected in three experiments as indicated in table 1. The esophageal muscle sheath had prominent striations, so the data from it were classified as striated muscle. An area of the colon was slit longitudinally, the mucosa was scraped away, and the remaining muscle was cut free. Histological examination showed only small amounts of mucosa remaining attached to the smooth muscle layers.

Results. Tissue phosphate. In table 1, PTH-treated rats had significantly lower inorganic phosphate in striated muscles but not in cardiac muscle or smooth muscle. This difference between cardiac and striated muscle was not significant since analysis of variance[6] gave a non-significant tissue-by-treatment interaction. When the acid soluble phosphate values were pooled, there was a significant reduction in the PTH groups ($p = 0.01$). This simultaneous reduction in acid soluble and inorganic phosphate indicates that the inorganic phosphate either was released from the muscle cell or was incorporated into a perchloric acid precipitable form. It rules out incorporation of inorganic phosphate into low molecular weight esters such as ATP, creatine phosphate or hexose phosphates. While some inorganic phosphate was released from liver and lung, most of the reduced phosphate content comes from the esterified fraction as shown by the larger drop in acid soluble phosphate.
^{32}P specific activity. Viscera studied earlier and erythrocytes rapidly exchanged inorganic phosphate between their cells and the plasma[7]. This rapid exchange was also true of the liver, lung, ventricle and, to a lesser extent, smooth muscle as shown by the closeness of their inor-

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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.

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Ovarian function in adult rats treated with antithymocyte serum

A. Bukovský, J. Presl, Z. Krabec and T. Bednařík

Institute for the Care of Mother and Child, nábř. K. Marxe 157, CS–147 10 Praha 4-Podolí (Czechoslovakia), 30 July 1976

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,

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