these cells. This assumption is, however, contrary to a previous suggestion that reticulocytes have 2, 3-DPG concentrations lower than those of mature red blood cells[13].

What is the significance of the 6- to 8fold rise in 2, 3-DPG in the red blood cells of sheep during anaemia? The answer to this question is obscure, although Professor G. J. Brewer (personal communication) argues that while 2, 3-DPG levels may be low and the affinity of the haemoglobin for 2, 3-DPG be reduced in animals such as sheep, the affinity is *not* zero and an increase in 2, 3-DPG will have some effect on oxygen affinity. His argument seems to be strengthened by the in vitro observations of Bunn et al.[7]. Despite a multitude of studies there is, as yet, no complete understanding of the importance of high concentration of 2, 3-DPG in the red blood cells of some species. It is known to affect pH and the Donnan equilibrium between the red cell and plasma[4,5]. Some workers have postulated that red cell 2, 3-DPG plays a role in potassium transport, possibly by way of 2, 3-diphosphoglycerate phosphatase[14,15]. It is of interest to note that the immature red cells in the blood of anaemic sheep have many times higher concentrations of potassium and a much greater activity of sodium potassium activated adenosine triphosphatase enzyme than the mature (low potassium) red cells[9]. It is apparent that further evaluation of the possible mechanisms relating hypoxia, red blood cell glycolytic rate and concentration of 2, 3-DPG is required.

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Testicular Leydig cells and Δ^5-3β-hydroxysteroid dehydrogenase in cadmium-treated toads (Bufo melanostictus)

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Summary. Injection of cadmium chloride in toad increased Leydig cell size (area) and Δ^5-3β-hydroxysteroid dehydrogenase activity in the testis.

Spermatogenic arrest due to cadmium injection in rat[2], mice[3], bird[4] and toad[5] has been noted by many investigators. Gunn et al[6,7] have reported that cadmium administration in rats and mice developes interstitial cell tumor (ICT) in testis. A single subcutaneous injection of cadmium salt also causes hypertrophy of the Leydig cells in pigeon[8]. To the best of our knowledge, the effect of cadmium on the Leydig cells of hibernating amphibians has not yet been studied.

The present work has been taken up to study the histology of the Leydig cells and the activity of enzyme Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) in cadmium-treated toad testis.

15 male toads (Bufo melanostictus) of average weights from 55 to 60 g were used in the present experiments

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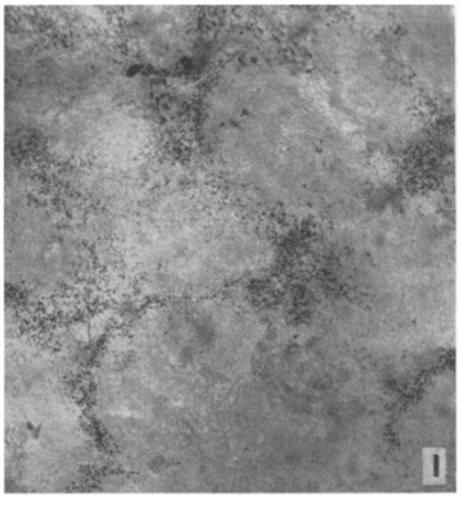

Fig. 1. Δ^5-3β-hydroxysteroid dehydrogenase in the testis of normal toad. × 96.

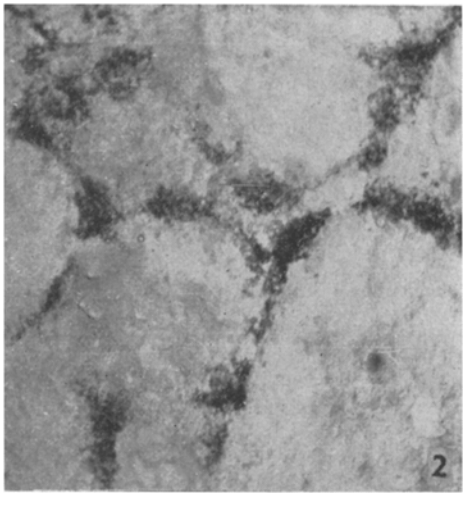

Fig. 2. Δ^5-3β-hydroxysteroid dehydrogenase in the testis of cadmium-treated toad. ×96.

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]

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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.