TABLE 1

Comparative measurements (in mm.) of Dorcatherium.

Measurement		Present specimen PUA 109/80D	*D. majus* A. M. No. 19520	*D. minus* A. M. No. 19365	*D. nagrii* G. S. I. 18079
Max. mesio-distal diameter	M_1	9	14		6.5
	M_2	11.6	17	13	6.6
Max. bucco-lingual diameter	M_1	6	9		3
	M_2	7	10.5	7.5	3

TABLE 2

Comparative measurements (in mm.) of Giraffokeryx.

Measurement	Present specimen PUA 103/80D	A. M. No. 19323
Max. mesio-distal diameter	33	33
Max. bucco-lingual diameter	18	17

molar, which has not completely cut the gum and is still erupting, exhibits rugose enamel. The measurements of this specimen are given in table 2.

Gazella sp. (figure H)

Description:– The specimen under description is the basal half (PUA 104/80D) of a horn-core of some gazellinae and was also recovered from the same locality as of the previous specimens. On the whole the horn-core is oval in cross-section with a flat inner side and a convex outer side. The surface of the horn-core is marked with shallow grooves which impart a rough appearance to it. The pedicle is devoid of grooves and is smooth. On the distal side of the horn-core a shallow vertical groove can be observed which gradually widens from the base to the free end. As such, the horn-core depicts a slight antero-posterior bending, making it covex anteriorly and concave posteriorly (figure E–G).

The available fossil fauna from this area does not permit clear-cut generalizations regarding the geological age of these sediments. The range of *Giraffokeryx* is from Chinji to Nagri Formation and that of *D. minus* is from Chinji to Dhokpathan. *Gazella* is known to occur even today. The occurrence of *Giraffokeryx*, however, restricts the upper age limit of these deposits to Nagri Formation. Even the lithological characters of these rocks are also of not much help. The lithological characters of the rock units of this area are more or less similar to the formations exposed in Ramnagar basin (J & K) and Nurpur-Ranital basin (H.P.), which have been considered to be Chinjis[1], and Upper Chinjis to lower Nagris[2], respectively. Pending further explorations to recover more fossil fauna, these deposits can be tentatively assigned to Chinji or Nagri.

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IN VITRO STUDIES ON INTERACTION OF CADMIUM CHLORIDE WITH ANGIOTENSIN CONVERTING ENZYME FROM RAT TESTES AND EPIDIDYMIS

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ANGIOTENSIN converting enzyme (ACE) was detected first by Cushman and Cheung[1] in the testes and epidi-

dymis of the rat, although its presence was reported in many tissues of the body[2]. Its role in the renin-angiotensin system which controls the aldosterone secretion is well established[3], but the localization of ACE in the male reproductive organs has not been elucidated except for our findings that this enzyme has a direct relationship with the testicular androgen production, spermatogenesis and the epididymal sperm maturation[4,5]. Treatment with cadmium chloride, which depresses steroidogenic and gametogenic function of the testes has shown to completely inhibit the testicular and epididymal ACE activities[5]. The present *in vitro* study was made to investigate if cadmium chloride can inhibit the ACE activity of testes and epididymis directly without the involvement of testosterone and sperm maturation, since cadmium has been already shown to inhibit testicular carbonic anhydrase[6] and amino-peptidases[7].

A 10% homogenate of testes and epididymis from normal male albino rats were prepared in 0.02M potassium phosphate buffer pH 8.3. Testes and epididymis homogenate were treated with different concentrations of cadmium chloride at room temperature (25° C) for 18 hr. After the treatment the ACE activities in the treated and the untreated samples were estimated by the method of Cushman and Cheung[8] using synthetic hippuryl-L-histidyl-L-leucine as substrate. The hydrolysis of hippuric acid from the substrate was measured; 0.1 ml of the homogenate was added to 0.3 ml of incubation medium and incubated at 37° C for 30 min. The final concentrations in assay medium were as follows: 5 mM NaCl, 100 mM potassium phosphate buffer of pH 8.3 and enzymatic protein. The reaction was stopped by adding 0.5 ml of 1N HCl. The hippuric acid was extracted with 2.5 ml of ethyl acetate by vortex mixing for 90 sec. One ml of the extract was evaporated to dryness at 120° C for 30 min. and the residue was taken up in 5 ml of water. The hippuric acid concentration was determined by measuring the absorbance at 228 nm against a zero time blank prepared by adding 1N HCl before the enzyme. The enzyme activity was linear with protein concentration and incubation time. Protein was estimated by the method of Lowry *et al*[9].

One unit of ACE activity is defined as the amount of enzyme that hydrolyses one μmol of hippuryl-L-histidyl-L-leucine per min at 37° C under standard assay conditions. The specific activity has been expressed as milliunits/mg protein.

The data were statistically analyzed and the test of significance was made as per students *t* test[10].

In vitro study of testes revealed that cadmium chloride when added to the medium at a concentration of 5 mg/ml, reduced the ACE activity significantly

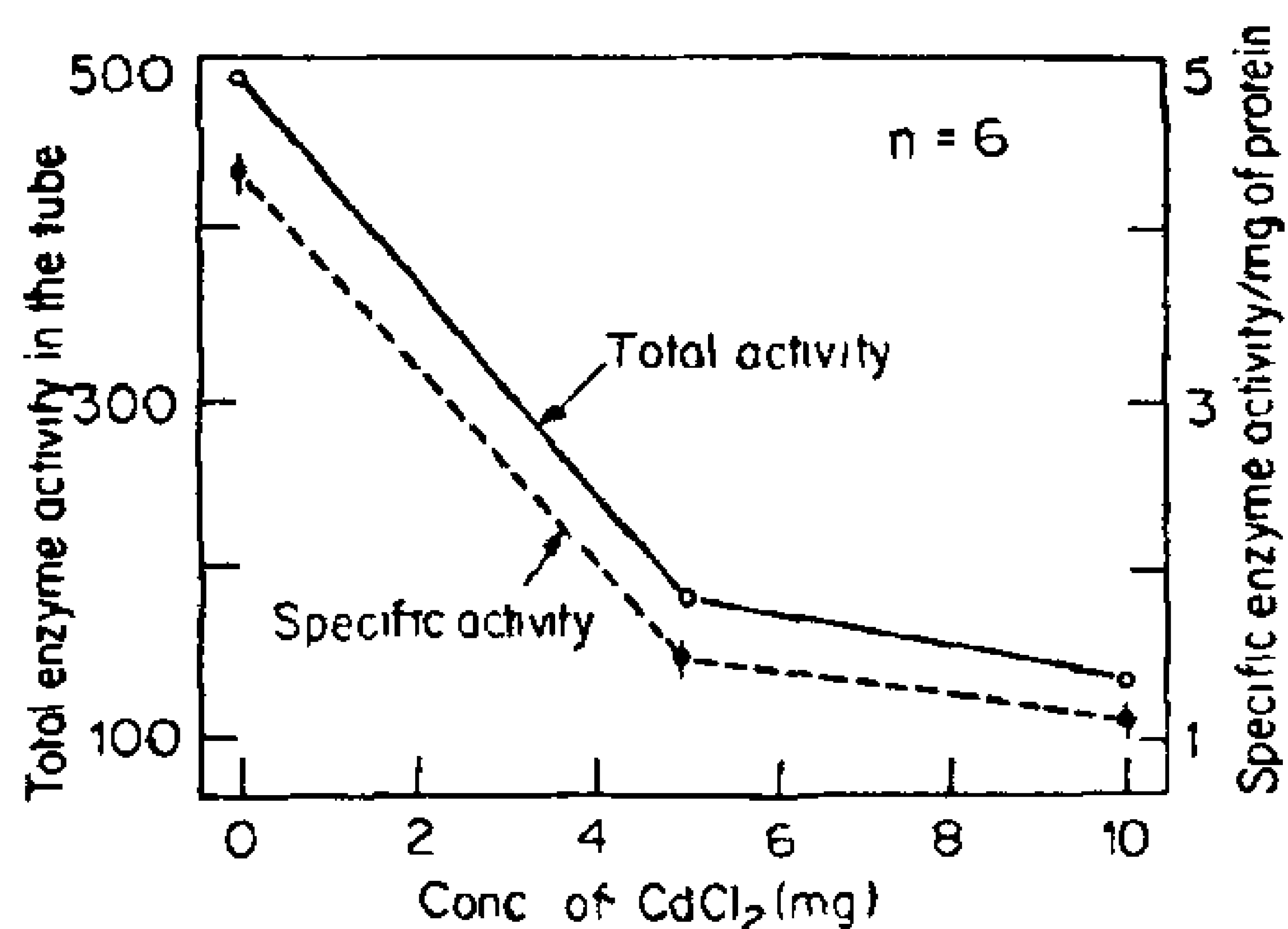

Figure 1. *In vitro* effect of $CdCl_2$ on testicular angiotensin converting enzyme (ACE). Reaction mixture = 2 ml at 25° C.

($P<0.01$) compared to control, which was more significant when the concentration of cadmium chloride was 10 mg/ml (figure 1). Similar observations were noted from the study with the whole epididymis Though the inhibition of ACE was significant ($P<0.01$) at concentrations of 3.75 mg/ml and 7.5 mg/ml, compared to controls (figure 2) the rate of

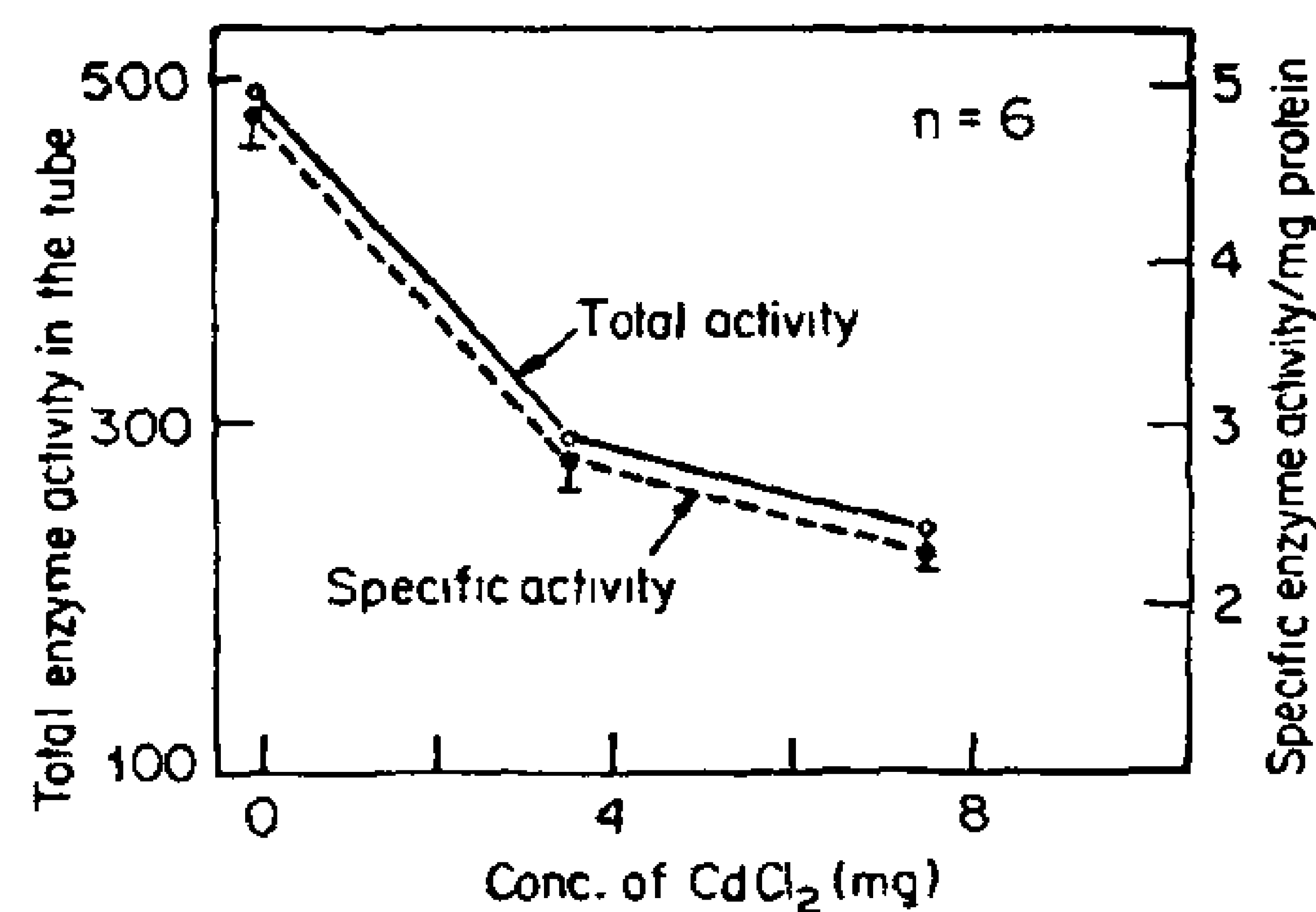

Figure 2. *In vitro* effect of $CdCl_2$ on epididymal angiotensin converting enzyme. Reaction mixture = 1.5 ml at 25° C.

decline was significantly ($P<0.05$) lower in the epididymis than that in the testes indicating more pronounced effect of cadmium on testicular function.

Johnson and Walker[6] have demonstrated that carbonic anhydrase activity in the testes of rat and fowl is inhibited by cadmium. The degenerative changes caused by cadmium in the testicular seminal elements are directly proportional to the reduction in the aminopeptidase activity[7]. The present study being the first one to report the effect of cadmium on the ACE activity of both the testes and epididymis, indicated that cad-

mium produces its deleterious effects on the testicular and epididymal functions by inhibiting the ACE and other enzymes and also by disturbing testicular steroidogenesis, sperm production and indirectly thereby epididymal sperm maturation. It is seen that the effect of cadmium on the ACE activity in these tissues does not follow a linear concentration-response relationship The mechanism of the inhibition of ACE by cadmium is under investigation.

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ACCUMULATION OF HISTIDINOL PHOSPHATE IN COPPER TOXIC CULTURES OF *NEUROSPORA CRASSA*

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EARLIER studies on copper toxicity in *Neurospora crassa* from this laboratory have shown that the activity of histidinol phosphate phosphatase (EC 3.1.3.15) was lowered in copper toxicity when the mould was cultured on a medium containing ammonium salts as the sole nitrogen source[1]. Attempts have been made to characterise the accumulated imidazoles in such conditions leading to the identification of histidinol phosphate as an important component of the accumulated imidazoles in copper toxicity. The experimental details and the results are discussed below.

Organism and growth conditions: N. crassa Em 5297 was grown for 72 hr on 10 ml of basal medium containing ammonium tartrate (0.1%) and ammonium nitrate (0.2%) as the nitrogen sources. Copper as $CuSO_4 5H_2O$) (1 mg/10 ml) was included in the medium aseptically to obtain 50% growth inhibited cultures. Similarly manganese as $MnSO_4H_2O$ (2 mg/10 ml) was included in some flasks to counteract the toxicity of copper and restore the full growth. Growth conditions and harvesting of mycelial pads were as described earlier[1].

Extraction of imidazoles: Mycelial pads were extracted for imidazoles according to Ames *et al*[2]. Three-day old mycelia were washed with cold water and then homogenised with hot water (5 ml/200 mg wet wt of mycelia). The homogenates were kept in boiling water bath for 10 min and the supernatants were collected by centrifugation. The residue was reextracted with 2 ml of hot water and the extracts pooled. The extract thus adjusted to pH 8.5 with saturated $Ba(OH)_2$ and the precipitate formed overnight was discarded by centrifugation. To the clear supernatant, 25% alcoholic $HgCl_2$ (0.02 ml/ml) was added to precipitate the imidazoles. The dried precipitate was dissolved in 0.1 ml of 0.5 N HCl and left overnight. Excess mercury was removed by passing H_2S and the clear supernatant was used for quantitating the histidinol phosphate.

Quantitation of histidinol phosphate by TLC: The extracts obtained as above were subjected to thin layer chromatography on glass plates coated with cellulose in single dimension using *n*-butanol:ethanol:ammonia (sp.gr. 0.88): water (10.1.2.4) and the imidazoles were detected by a diazonium spray reagent[3]. Histidinol, histidine and histidinol phosphate were used as markers and were noted to have an R_f value of 0.6, 0.26 and 0.06 respectively in the solvent system. The area corresponding to histidinol phosphate was scrapped off, eluated with 20% ethanol and again subjected to separation in the same solvent system. Histidinol phosphate in the eluate was quantitated by the diazotisation procedure employing *p*-nitroaniline[4]. The values were corrected for recovery of histidinol phosphate which was also determined with standard histidinol phosphate (recovery was 98.5%).

Table 1 depicts the histidinol phosphate content in extracts obtained from control, copper toxic and manganese supplemented cultures. It is seen that the