Table I. Alteration of the sex ratio of offspring born to BALB/cDg mice infected with the lactate dehydrogenase virus

Items	Mice Experimental[a,b]	Control[c]
Litters	36	41
Babies	228	268
Babies/litter	6.3	6.5
Weanlings	126	140
Weanlings/litter	3.5	3.4
Male offspring	85	81
Female offspring	88	117
Male: female sex ratio (%)	49:51[d]	41:59

[a] Infected for 10 to 19 days before conception. [b] A total of 55 offspring were not sexed. [c] A total of 70 offspring were not sexed. [d] P 0.12.

Table II. Comparison of plasma lactate dehydrogenase (LDH) levels in the BALB/cDg and C57BL/Fg strains of mice

Treatment	No. of mice tested per group	Plasma LDH (units/ml)			
		BALB/cDg		C57BL/Fg	
		Range	Mean	Range	Mean
Infected[a]	35	2400–5700	4150	2400–5400	4050
Noninfected	50	200–1200	700	300–1300	750

[a] Plasma samples collected 1 week after an i.p. injection (0.1 ml/mouse) of $10^{5.0}$ ID_{50}/ml of the LDH virus.

normal level of plasma LDH; or 2. the degree of increase in enzyme activity following infection with the LDH virus.

Table III shows the results obtained in the second experiment. It will be noted that spermatozoa LDH levels in normal BALB/cDg males were 3 times greater than those in infected animals. However, enzyme levels in similar preparations from infected and noninfected C57BL/Fg males were essentially the same. These data, then, may be said to offer partial support for the hypothesis that the alteration in sex ratio is related to the level of spermatozoa LDH. Beyond this, and until such time as further information becomes available, we can only suggest that alternative explanations also be explored.

Table III. Comparison of lactate dehydrogenase (LDH) levels in spermatozoa from normal and infected mice of the BALB/cDg and C57BL/Fg strains

Treatment	No. of mice tested per group	Spermatozoa LDH (units/ml of extract)			
		BALB/cDg		C57BL/Fg	
		Range	Mean	Range	Mean
Infected[a]	15	110–630	275	120–550	285
Noninfected	14	390–1660	775	110–730	275

[a] Sperm samples were collected 2 weeks after an i.p. injection (0.1 ml/male) of $10^{6.5}$ ID_{50}/ml of the LDH virus.

Zusammenfassung. Durch Infektion mit LDH-Virus wird bei Mäusen, deren Nachkommen normalerweise ein Geschlechtsverhältnis < 1 aufweisen, ein solches von 1 erreicht. Da sich die Infektion der Eltern beider Stämme nicht in einer unterschiedlichen Zunahme der Serum-LDH, wohl aber in einer solchen der Spermien-LDH auswirkt, wird angenommen, dass die LDH-Aktivität der Spermien sich in einer zwar noch unbekannten Weise auf das Geschlechtsverhältnis der Nachkommen auswirkt.

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A New Type of Acid Phosphatase from Rat Testis

At least three enzymatically distinct forms of acid phosphatase have been separated from various mammalian tissues[1–3]. They have different substrate specificities, different responses to inhibitors and different tissue and subcellular localizations. Histochemical studies have indicated that acid phosphatase is mainly associated with lysosomal particles[4]. Other sites of reaction are cisternae of smooth endoplasmic reticulum[5] and Golgi apparatus[6,7]. Similar localization has been demonstrated for acid phosphatase in testicular Leydig[8] and Sertoli cells[9] and additionally in acrosomes of spermatozoa[10,11].

The present study describes four distinct acid phosphatases in rat testicular tissue with special emphasis to a new soluble enzyme not previously described in other tissues.

Material and methods. Adult albino rats were sacrificed by rapid decapitation, their testes and samples from 9 other tissues were taken and homogenized at 4 °C either in 0.25 M sucrose solution or in 0.02 M *Tris*-HCl buffer pH 7.5 with a glass homogenizer and teflon pestle. Samples in 0.25 M sucrose were centrifuged with $105{,}000 \times g$ for 1 h and the soluble and particle fractions were diluted with 0.02 M *Tris*-HCl, pH 7.5, for enzyme assays. Protein was determined by the method of LOWRY et al.[12].

Tissue samples homogenized in *Tris*-HCl buffer were sonicated for 30 sec (MSE Ultrasonic Disintegrator), applied on a DEAE-cellulose (Whatman DE 23) column (2×30 cm) and eluted with a continuous NaCl gradient (0–0.35 M) in 0.02 M *Tris*-HCl buffer pH 7.5. Aliquots of 5 ml were collected. The protein content was determined by the absorbancy at 280 nm.

Acid phosphatase activity of total homogenate, soluble and particulate fractions and chromatographic fractions were assayed at 37 °C with *p*-nitrophenyl phosphate

(Sigma Chem. Co.) as subtrate. The hydrolysis rate was estimated as nmole *p*-nitrophenol liberated/mg protein/min. Various modifier substances of analytical grade were incubated with the enzyme 30 min before the addition of substrate at concentrations indicated.

Results. The hydrolysis rate of *p*-nitrophenyl phosphate at pH 5 by total homogenate of different rat tissues are shown in Table I. Testicular tissue had one of the highest activities toward this substrate. A marked variation was also found in the amount of soluble acid phosphatase activity. Testicular tissue had more than $^2/_3$ of the total activity in soluble form, while the prostate gland had about 90% bound to particles after identical homogenization procedure.

The effect of Ni^{2+} (1 m*M*), Mn^{2+} (1 m*M*), NaF (10 m*M*) and Cu (0.5 m*M*) on the hydrolysis of *p*-nitrophenyl phosphate is shown in Table II. It is particularly evident that Ni^{2+} and Mn^{2+} caused a stimulation of hydrolysis in the soluble testicular fraction without any comparable effect in other tissues. In most cases NaF was highly inhibitory, particularly for the particle fractions. As a marked contrast, it caused a slight stimulation of hydrolysis in the testicular soluble fraction. The particle-bound enzyme activity was inhibited from 36–66% by Cu^{2+}. The soluble enzyme activity was still more sensitive to this metal ion and the soluble testicular activity was almost totally abolished.

Fractionation of total testicular homogenate with DEAE-cellulose chromatography resulted in 4 different hydrolytic areas for *p*-nitrophenyl phosphate in 0.1 *M* acetate buffer, pH 5.0 (Figure 1). In the presence of Ni^{2+} no change was observed in enzymes I–III, while activity IV was stimulated more than twice. NaF (10 m*M*) caused a total inhibition of enzyme I, about 70% inhibition of enzyme II, a slight inhibition of enzyme III and a small but detectable stimulation of enzyme IV. Cu^{2+} had some inhibitory effect on enzyme I, no effect on enzymes II and III, but totally inactivated enzyme IV.

The determination of pH-optimum in 0.2 *M* acetate buffer series gave maximum activity for enzyme I at pH 5, for enzyme II at pH 5.7, for enzyme III at pH 5.4 and for enzyme IV at pH 6 (Figure 2).

Table I. Hydrolysis of *p*-nitrophenyl phosphate by total homogenate of different rat tissues and percentage of hydrolytic activity present in the soluble fraction after centrifugation at 105,000 × *g* for 1 h in 0.25 *M* sucrose solution

Tissue	Total activity (nmole/mg/min)	Amount of activity in soluble fraction (%)
Epididymis	111.6	28.9
Spleen	101.9	44.3
Testis	101.7	68.4
Kidney	75.9	45.9
Liver	59.5	61.2
Intestine	50.3	35.5
Adrenal	47.3	17.6
Submandibular	44.2	19.8
Prostate	43.4	10.8
Pancreas	27.9	30.8

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Table II. Change in hydrolysis rate of *p*-nitrophenyl phosphate from control value (100%) of soluble (Sol.) and particle (Par.) fractions of different rat tissues in the presence of 4 modifier substances

Tissue	Ni^{2+} (1 m*M*)		Mn^{2+} (1 m*M*)		NaF (10 m*M*)		Cu^{2+} (0.5 m*M*)	
	Sol.	Par.	Sol.	Par.	Sol.	Par.	Sol.	Par.
Epididymis	103.5	113.4	76.4	86.1	46.7	46.5	28.0	66.4
Spleen	93.8	101.0	57.3	57.2	27.8	10.9	32.6	47.6
Testis	207.9	144.1	157.9	90.3	115.8	15.3	0.9	46.6
Kidney	103.3	114.9	98.2	83.1	63.9	10.3	21.6	54.7
Liver	101.9	96.3	86.8	52.9	84.6	6.4	9.2	66.6
Intestine	108.2	112.4	100.5	83.8	43.6	13.8	25.0	58.6
Adrenal	94.7	125.8	96.5	81.5	71.9	4.0	14.0	56.5
Submandibular	124.5	101.3	86.8	71.2	9.4	3.9	50.0	56.6
Prostate	103.5	107.4	79.0	21.4	75.4	7.7	15.8	35.9
Pancreas	101.2	102.6	57.5	49.0	37.9	1.9	29.5	60.5

Discussion. The plurality of acid phosphatases has been demonstrated in many tissues, and enzymes I–III in rat testicular tissue showed closely similar characteristics to the 3 previously described enzyme activities in human placenta[2] and bovine liver[3]. More detailed studies may, however, show differences between those present in different organs and different animal species. The 4 testicular acid phosphatases are clearly distinct enzymes and not only different molecular forms of one enzyme, since they differ from each other in their pH-optima, inhibitor and activator characteristics and also in their substrate specificity (unpublished observations). In contrast to this, the numerous isozymic forms of prostatic acid phosphatase in electropherogram have similar characteristics and differ from each other only in the sialic acid content of the molecule[13].

The stimulation of *p*-nitrophenyl phosphate hydrolysis by Ni^{2+} and Mn^{2+} was particularly characteristic for enzyme IV. In marked contrast for the other enzymes, it was not inhibited by NaF, which is known to be inhibitory to all the previously described acid phosphatases[2] and was also to enzymes I–III in testicular tissue. The slight stimulation of enzyme IV activity by NaF remains to be confirmed with a more purified enzyme preparation.

Of particular interest was the presence of acid phosphatase IV in testicular tissue, since studies with 9 other rat tissues did not reveal a similar enzyme elsewhere. Preliminary studies with isolated seminiferous tubules also showed this activity. This may suggest that enzyme IV is functionally related to spermatogenesis. The present study did not show, however, the precise cellular or subcellular site for enzymes I–III, but they seem to be connected to particles, whereas enzyme IV is clearly in soluble form. Further studies are necessary to reveal the relationship of these biochemical findings to the previously reported histochemical observations[14].

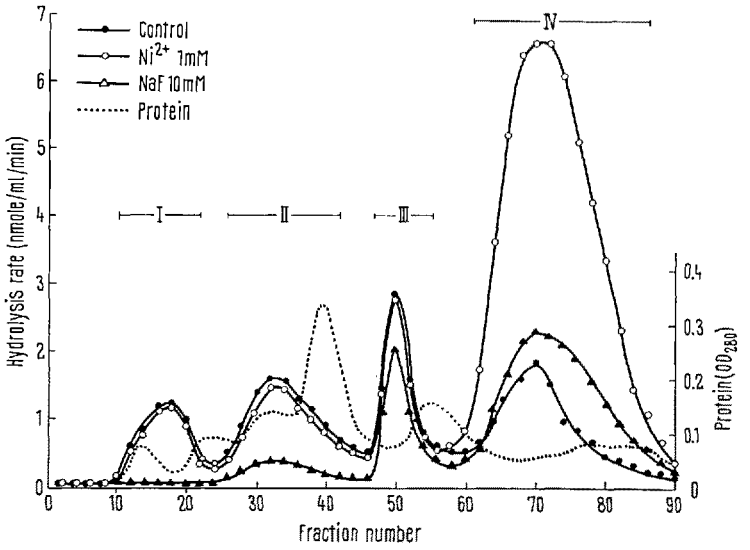

Fig. 1. Hydrolysis of *p*-nitrophenyl phosphate at pH 5.0 by DEAE-cellulose chromatography fractions of rat testicular homogenate with and without some modifier substances as indicated.

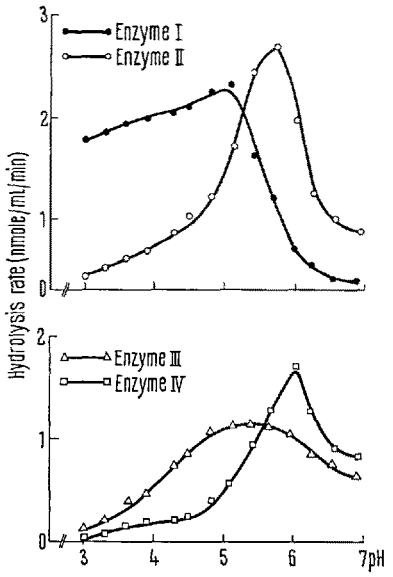

Fig. 2. Effect of pH on the hydrolysis rate of *p*-nitrophenyl phosphate by the 4 enzyme activities separated by DEAE-cellulose chromatography as shown in Figure 1.

Zusammenfassung. Vier verschiedene Typen saurer Phosphatase wurden mit DEAE-Zellulose Chromatographie von Rattenhoden separiert. Drei davon waren mit denjenigen anderer Gewebe nahe verwandt. während der vierte Typ löslich war und mit Ni^{2+} und Mn^{2+} aktiviert wurde (pH 6) und mit einer Fermentaktivität, die in 9 anderen Geweben der Ratte nicht zu finden war.

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