PHOSPHATASES IN CHICKEN SEMEN*

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Summary. Chemical analysis has revealed that the seminal plasma of the chicken contains high levels of acid phosphatase and low levels of alkaline phosphatase. There were significant differences in the acid phosphatase levels of individual males, but not of different breeds. Analysis of various sections of the male reproductive system demonstrated high levels of the acid phosphatase in the vas deferens and epididymis, and lesser but substantial amounts in the other sections tested.

INTRODUCTION

The semen of mammals contains high concentrations of acid phosphatase. No chemical analyses have been made for the presence of this enzyme in chicken semen, although Lake (1957a) has observed that feeble staining reactions for acid phosphatase are found in some areas of the male chicken's reproductive tract. This study was conducted to establish the level of acid phosphatase in chicken semen and to determine its site of production. The acid phosphatase in mammalian semen is produced by the prostate gland (Mann, 1954) but this structure is absent in the chicken (Lake, 1957a). Chemical analyses for alkaline phosphatase were also included, since it is found in high concentration in bull and ram semen (White, 1958).

MATERIAL AND METHODS

Males of three different breeds (White Leghorn, Red Vantress and a Flightless strain) were trained for several days before use, and individuals producing the largest quantity of semen were selected. Semen was collected by a one-man technique (Bogdonoff & Shaffner, 1954), centrifuged at 1200 *g* for 10 min, and the supernatant analyzed the same day for acid and for alkaline phosphatase by the methods of Andersch & Szczypinski (1937) and Bessey, Lowry & Brock (1946), respectively. Semen was collected from the same males 3 days later and the analyses repeated.

In a separate experiment, various sections of the male reproductive tract were analyzed for acid phosphatase in White Leghorn males. After sacrifice by

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dislocation of the neck vertebrae, the different parts of the reproductive system were dissected out, homogenized in nine parts of distilled water with a tissue grinder held in an ice water bath, and the entire homogenate or appropriate dilutions thereof analyzed on the same day for acid phosphatase. In order to reduce turbidity from insoluble particles, the procedure of analysis was modified at the end of the incubation period according to the method of Nimni (1957). No attempt was made to remove the contents of the various parts dissected out. Just before sacrifice of the male, blood was taken by heart puncture and semen collected. The semen was centrifuged and its plasma removed, after which the packed spermatozoa were diluted 1 to 10 with a sodium-chloride solution (9.9 g/l) and centrifuged again. After three washings, the packed spermatozoa were diluted 1 to 10 with distilled water and homogenized.

TABLE 1
ACID PHOSPHATASE CONTENT OF SEMINAL PLASMA OF
SEVERAL BREEDS

<i>Breed</i>	<i>mM nitrophenol/l/hr</i>				<i>Av.</i>
	<i>Male No.</i>				
	1	2	3	4	
Flightless	685	825	825	1040	844
Red Vantress	365	1060	600	485	628
White Leghorn	655	1050	990	215	728

RESULTS

Results as shown in Table 1 indicate a high level of acid phosphatase in the seminal plasma of the chicken. There were highly significant differences between males of the same breed but not between breeds. When the average value is converted into King-Armstrong units by multiplying by 4.5, the value

TABLE 2
ALKALINE PHOSPHATASE CONTENT OF SEMINAL PLASMA
OF SEVERAL BREEDS

<i>Breed</i>	<i>mM nitrophenol/l/hr</i>					<i>Av.</i>
	<i>Male No.</i>					
	1	2	3	4		
Flightless	2.6	1.8	5.2	3.6	3.3	
Red Vantress	1.8	1.9	3.3	1.9	2.2	
White Leghorn	2.5	1.5	2.7	1.6	2.1	

obtained of approximately 3300 units/100 ml is between the average values obtained for man and the bull (White, 1958). The values for alkaline phosphatase (Table 2) were low and somewhat variable. Thus, the seminal plasma of the chicken resembles that of man in which the level of acid phosphatase is

higher than that of alkaline phosphatase, and is different from that of the bull in which the reverse is true (White, 1958).

Results on the acid phosphatase content of different components of the male reproductive system (Table 3) reveal high levels of the enzyme in the epididymal region, anterior and muscular vas deferens, as well as the seminal plasma. The testis, phallus, lymph folds, vascular bodies and washed spermatozoa contain substantial but lower amounts of the enzyme. The level in the blood serum is extremely low.

TABLE 3
ACID PHOSPHATASE CONTENT OF COMPONENTS OF THE
MALE REPRODUCTIVE SYSTEM

Fluid, tissue or organ	mM nitrophenol/l or kg/hr			
	Male No.			Av.
	1	2	3	
Seminal plasma	320	700	460	493
Washed spermatozoa	28	46	40	38
Blood serum	2.0	0.6	0.8	1.1
Testis	231	226	277	245
Epididymal region	520	681	370	524
Anterior vas deferens	527	958	597	694
Muscular vas deferens	620	595	609	608
Accessory glands:				
Phallus	89	78	58	75
Lymph folds	162	154	151	156
Vascular body	231	293	228	251

DISCUSSION

It is not clear from these results where the acid phosphatase of seminal plasma is produced. More refined techniques are necessary for clarification of this point, especially measurements of the level of the enzyme in the extracellular fluid in the lumen of various sections of the reproductive tract.

However, it appears likely that most, if not all, of the seminal acid phosphatase has been produced by the time the spermatozoa leave the vas deferens, since the highest level observed was in the vas deferens. The accessory organs apparently add little if any of this enzyme to seminal plasma, since the level in the seminal plasma is not raised above that in the vas deferens. This agrees with the findings of Lake (1957a) that the chicken does not contain a structure equivalent in function to the mammalian prostate gland. The secretions of the accessory glands may possibly serve to dilute the acid phosphatase in semen.

The alkaline phosphatase was found to be present in low concentrations; this can be explained, at least partially, as originating from the blood as a transudate in the process of artificial collection of semen, since Lake (1957b) has found this to occur. It is highly unlikely, however, that significant amounts of acid phosphatase in semen originated from this source, since the level of this enzyme in serum is quite low.

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Note added in proof

The presence of acid and alkaline phosphatase in chicken seminal plasma has recently been described by other workers [Bell, D. J. & Lake, P. E. (1960) *Biochem. J.* **75**, 15P]. Another paper from the same laboratory [Lake, P. E. (1960) *Biochem. J.* **75**, 15P] has demonstrated histochemically intense acid-phosphatase activity in the entire length of the vas deferens and parts of the epididymal region, and some activity in certain testicular cells. Both of these results are in essential agreement with the results of this investigation.