enzyme capable of hydrolysing isomaltodextrins of D.P. > 3; *Lb. bifidus* cells would, however, require such an enzyme to convert the products of the extracellular dextranase activity into glucose.

In view of the range of isomaltodextrins hydrolysed by the cell extracts we feel that the enzyme can justifiably be termed an isomaltodextrinase rather than merely an α -1 $\rightarrow$ 6-glucosidase. We think that it is the first enzyme of its kind to be reported.

SUMMARY

1. Cell extracts prepared from a rumen strain of *Lactobacillus bifidus*, grown on dextran, contain an α -1 $\rightarrow$ 6-glucosidase that readily hydrolyses isomaltodextrins of degree of polymerization 2-9 to glucose.

2. Hydrolysis of the isomaltodextrins is by the cleavage of single glucose units from the non-reducing end of the molecule.

3. Isomaltodextrins of degree of polymerization 2-5 are hydrolysed at the same rate; as the degree of polymerization of the isomaltodextrins progressively increases above 5 the rate of hydrolysis decreases.

4. The enzyme possesses optimum activity within the pH range 5.5-6.5, and temperature range 36-41°C; it may also act as a transferase if sufficient isomaltodextrin is present.

5. The extracts also hydrolyse nigerose and a variety of β -linked sugars but scarcely attack melibiose, maltose, turanose, leucrose, α -trehalose or methyl α -glucoside.

Thanks are due to Mr R. T. J. Clarke of this Laboratory for providing the freeze-dried culture, to Dr F. H. Stodola

(United States Department of Agriculture), Dr D. H. Hutson (Royal Holloway College, University of London) and to Dr M. J. Clancy for gifts of sugars, and to Miss P. Boyden for technical assistance.

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Tissue Components of the Domestic Fowl

5. PHOSPHOMONOESTERASES IN THE SEMINAL PLASMA OF THE COCK*

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(Received 4 August 1961)

During attempts to preserve fertile avian spermatozoa *in vitro*, it was found that cock seminal plasma possesses high acid-phosphomonoesterase activity (acid phosphatase). There was also a little alkaline-phosphatase activity. The former finding was somewhat surprising since birds are devoid of

those accessory reproductive organs that, in most mammals, are believed to contribute the bulk of the acid phosphatase(s) to the chemically different semen of the latter class (Mann, 1954). Lake (1960*b*, 1962) showed, concomitantly, that the acid phosphatase(s) of the cock was mainly derived from secretions of the vasa efferentia and vasa deferentia with a possible contribution from the

* Part 4: Bell (1960).

germinal epithelium in the testis. The present work reports an investigation of the phosphatase(s) activity of cock seminal plasma; a preliminary account has already been given (Bell & Lake, 1960).

MATERIALS AND METHODS

Seminal plasma. Uncontaminated semen, as defined by Lake (1957), was collected from Brown Leghorn males by massage and within 5 min. was centrifuged at 1500g for 20 min. at 5°. The separated plasma was kept at 5° for immediate analysis; after dilution it could be satisfactorily stored at -30° (see below).

Fluids from vasa deferentia and erectile cloacal tissue. Since Lake (1960b, 1962) had shown histochemically that the bulk of the acid phosphatase(s) was secreted by the vasa deferentia it was necessary to confirm this observation by analytical procedures. In three 1-year-old cocks, the junctions between the epididymal regions and the vasa deferentia were ligated. Three to four weeks later the contents of the vasa were milked out several times, by massage as for semen collection, until spermatozoa were no longer obtained. Afterwards, the secretions accumulating in the vasa were obtained, on several occasions, and analysed for phosphatase activity under the same conditions as for whole seminal plasma.

By fractional collection (Lake, 1957) the transparent fluid was obtained from the erectile lymph folds and external vascular tissue of the cloacae of normal adult cocks (12-18 months old) and ones with ligated vasa deferentia. The phosphatase activity of this secretion was also examined as it can easily contaminate the semen when the massage method of collection is used.

Blood plasma. Blood was taken from the wing vein with a heparinized syringe and immediately centrifuged at 5°. Undiluted plasma was used for both alkaline and acid phosphatase measurements.

Optimum pH of seminal plasma acid-phosphatase activity with 4-nitrophenyl phosphate as substrate. This was examined in the uncontaminated seminal plasma of 1-year-

old cocks by using 23 Michaelis acetate-veronal-HCl buffers (0.1M) over the pH range 2.92-7.40. Diluted (1:100) plasma (0.05 ml.) was mixed with ice-cold buffer (1.0 ml.), 66 mM-disodium 4-nitrophenyl phosphate (0.1 ml.) added, and the whole incubated at 37° for 1 hr. After cooling rapidly, 10 ml. of 0.1M-NaOH was added to each digest, and the colour that developed was measured at 397 m μ . Two flat peaks were observed at pH 4.60-4.95 and pH 5.15-5.30. With Michaelis buffers at intervals of 0.1 pH unit the peaks were again broad, but had maxima at pH 4.9 and 5.2. At pH 7.4 the enzymes showed no activity against 4-nitrophenyl phosphate. Acetate buffer gave identical results.

Dilution by 0.9% sodium chloride solution and by 'glutamate medium' on acid-phosphatase activity. Since the acid-phosphatase activity of the seminal plasma was so great it was necessary to work at high dilutions. Both 0.9% NaCl and a glutamate-containing saline fluid, based on a partial analysis of the protein- and lipid-free uncontaminated seminal plasma (Lake, 1960a), were examined as diluents. No difference was detected between the two media; for subsequent experiments the seminal plasma was diluted 50-fold with 0.9% NaCl immediately after collection. The acid-phosphatase activity remained stable in the diluted plasma for at least 30 days at -30°. On the other hand, a progressive decline in the alkaline-phosphatase activity of the undiluted plasma was noted under the above conditions of storage.

Seminal plasma phosphatase activity with various substrates and in the presence of inhibitors. Alkaline-phosphatase activity in undiluted blood and seminal plasmas was measured at pH 10.08 (Na₂CO₃-NaHCO₃ buffer) with 4-nitrophenyl phosphate as described by Bell (1960) for blood plasma.

Acid-phosphatase activity of the various fluids, suitably diluted, was measured with 4-nitrophenyl phosphate at pH 4.9 in citrate buffer by an application (Bell & Siller, 1962) of the original principles of Andersch & Szczypinski (1937). The reaction mixture was finally diluted with M-NaOH; 10-25 ml. was normally used. Units of both acid- and alkaline-phosphatase activities are expressed as

Table 1. Average values of acid- and alkaline-phosphatase activities in the seminal plasma and associated fluids of the domestic cock

Activity is expressed as mg. of 4-nitrophenol liberated in 1 hr. by 100 ml. of fluid at 37°.

Material	Phosphatase activity	
	Acid (seminal plasma diluted 1 to 50) 0.2M-citrate, pH 4.9	Alkaline (undiluted plasma) 0.1M-Na ₂ CO ₃ / NaHCO ₃ , pH 10.08
Seminal plasma (30 cocks)		
Nov. 1959	10 600	37
Dec.	15 300	
Jan. 1960	19 000	
Feb.	15 000	
Mar.	16 200	
May beginning	6 300	
May end	4 800	
Vas deferens fluid	13 000	56
Cloacal gland fluid	1620, 5800, 3500*	41, 20*
Blood plasma (undiluted)	5	43

* Vasectomized males.

mg. of 4-nitrophenol liberated by 100 ml. of plasma in 60 min. at 37°.

The cock acid-phosphatase activities with glycerol 1- and 2-phosphate, bis-(4-nitrophenyl) phosphate and 4-nitrophenyl phosphate as substrates were compared on the basis of the orthophosphate liberated (cf. Hawk, Oser & Summer-son, 1947). The inorganic phosphate was determined by the method of Allen (1940). Acetate buffer was used in these tests since the citrate ion (0.2M) interfered with the phosphate determination (cf. Abul-Fadl & King, 1949).

In the study of inhibitors both citrate and acetate buffers (pH 4.9) were examined, as Abul-Fadl & King (1949) showed that the nature of the organic anion could affect the activity of the multivalent cation. Both buffers gave identical results with cock plasma in the absence of inhibitory substances. The effects on cock acid phosphatase of Cu^{++} and Ca^{++} ions, dextro- and laevo-rotatory tartrates and F^- ions were studied and compared with their action on the human seminal plasma enzyme and on a preparation of human prostatic acid phosphatase. The former was collected into glass from healthy males, immediately centrifuged as for cock semen and diluted 1000-fold with 0.9% NaCl. The prostatic extract was kindly provided by Mr I. E. Lush, who prepared it by the method of Schmidt (1955) from fresh, enucleated, hypertrophic glands obtained from the Royal Infirmary, Edinburgh.

RESULTS

Acid phosphatase. Uncontaminated semen samples were collected at intervals during 6 months from 30 Brown Leghorn cocks. The average levels of acid-phosphatase activity (against 4-nitrophenyl phosphate) are given in Table 1. There is an apparent seasonal decline in activity; this may be due to increasing age of the birds.

The acid-phosphatase activity in the spermatozoan-free secretion of the vasa deferentia was high and of the same order as that found in the

whole seminal plasma. There was a moderate amount of activity in the fluid transudate and secretions of the erectile cloacal tissues which agrees with the histochemical observations of Lake (1962). During collection slight contamination by vas deferens fluid took place and it may be that, were it possible to obtain absolutely uncontaminated cloacal gland secretion, its true level would be lower still. In Table 1 the average level of blood plasma acid-phosphatase activity in the cock is also reported. Bell (1961) surveyed fowls at different ages and all of these, normal females as well as males, show a small activity of the same order as found in the cock.

Cock seminal-plasma acid phosphatase hydrolysed both the 1- and 2-phosphate of glycerol, being more active against the primary than the secondary ester (Table 2). However, the hydrolysis of 4-nitrophenyl phosphate was much more rapid than was that of the glycerol derivatives. Some acid-diphosphoesterase activity could be demonstrated by using bis-(4-nitrophenyl) phosphate as substrate.

A summary of inhibition experiments with cock and human acid phosphatase(s) is given in Table 3.

Table 2. *Relative activity of the acid phosphatase of cock seminal plasma towards various substrates*

Activity is expressed as mg. of inorganic P liberated by 100 ml. of seminal plasma in 1 hr. at 37° in acetate (0.2M) buffer (pH 4.9).

Substrate	Activity
Glycerol 2-phosphate	195
Glycerol 1-phosphate	374
4-Nitrophenyl phosphate	1400
Bis-(4-nitrophenyl) phosphate	55

Table 3. *Inhibition of acid-phosphatase activities against 4-nitrophenyl phosphate of seminal plasmas of cock and man and of human prostatic extract, by various added ions*

Conditions of enzyme assay: all assays were incubated for 60 min. at 37° with buffers of strength 0.2M, pH 4.9 (1 ml.), and 0.05 ml. of diluted plasma or organ extract.

Added ion, with final concn.	Buffer	Concn. of substrate	Percentage inhibition		
			Cock	Man	Human prostatic extract
Cu^{++} (1 mM)	Citrate	5.75 mM	12	63	20
	Acetate		75	87	66
Ca^{++} (100 mM)	Citrate		0	10*	11*
	Acetate		0	10*	11*
Dextrorotatory tartrate (1 mM)	Citrate		9	50	50
	Acetate		4	50	50
Dextrorotatory tartrate (10 mM)	Citrate	10.5 mM	0	79-97	80
	Acetate		0	86	82
Laevorotatory tartrate (10 mM)	Citrate		0	—	—
	Acetate		100	(97†)	(96†)
F^- (2 mM)	Citrate				

* These values refer to apparent stimulation, not inhibition.

† Abul-Fadl & King (1949), using phenyl phosphate in acetate buffer.

Sodium fluoride (2 mM) completely inhibited activity in all cases. Dextrorotatory tartrate (up to 10 mM) had no significant effect on the cock enzyme although it strongly depressed the activity of the acid phosphatase(s) of human semen and prostate. Cupric ions (1 mM) inhibited enzyme activity in all three cases; this was much more marked with acetate buffer. Calcium ions (100 mM) were without effect on the cock enzyme and showed a possible, very slight potentiation with the phosphatases of human origin in both citrate and acetate buffers. In separate experiments laevorotatory tartrate and Mg^{2+} ions were found to be without effect on the cock phosphatase.

Alkaline phosphatase. This was measured only in cock seminal plasma against the nitrophenyl substrate and was consistently low; it did not change notably with either advancing age or with the season. The average level of activity for the whole period of investigation is given in Table 1. The level in the blood plasma is of the order found in other cocks and non-laying hens (cf. Bell, 1960). Added Mg^{2+} ions potentiated the activity, Ca^{2+} ions had no effect and F^{-} ions were completely inhibitory.

DISCUSSION

The high acid-phosphatase activity in the seminal plasma of the cock confirms the histochemical findings of Lake (1960*b*, 1962), which showed an active secretion of acid phosphatase by the epithelium of the vasa deferentia and little alkaline-phosphatase activity in epithelial secretions of the genital tract. A correspondingly low level of alkaline-phosphatase activity was found, analytically, in the seminal plasma which could be correlated with the absence, in the cock, of accessory reproductive organs, particularly seminal vesicles. The marked acid-phosphatase activity in the absence of a prostate gland provides an interesting subject for future study in the field of comparative reproductive physiology. The independent work of Wilcox (1961) on cock seminal plasma has confirmed our own findings with respect to the high level of acid-phosphatase activity and the presence of a relatively weak alkaline phosphatase.

That the acid phosphomonoesterase produced in the genital apparatus of the cock is of a nature different from that of mammalian prostatic origin is shown in Table 3 where it is compared with the seminal and prostatic enzymes of man. The difference is especially marked with respect to the lack of effect of dextrorotatory tartrate, which, as shown by Abul-Fadl & King (1949), Anagnostopoulos (1953) and Tsuboi & Hudson (1955), almost completely inhibits the enzyme from the human prostate or seminal fluid. Bell & Lake (1962) have

reported elsewhere a similar inhibitory effect of this anion on the seminal acid phosphatases of several other mammals.

In our experiments 1 mM- Cu^{2+} had little effect (12% inhibition) on the cock acid phosphatase in citrate buffer but was markedly inhibitory (75%) in acetate. Parallel behaviour of Cu^{2+} ions in the two buffers was observed with the human enzymes. Abul-Fadl & King (1949), using 1 mM- Cu^{2+} ions in acetate buffer (pH 5) and phenyl phosphate as substrate, observed essentially the same results on human material but found a slight activation of the enzyme when citrate buffer (pH 5) was used. Tsuboi & Hudson (1955), working with highly purified prostatic acid phosphatase in acetate buffer, found the inhibitory effect (70%) of 2 mM- Cu^{2+} ions to be completely reversed by the presence of 50 mM-citrate. With crude enzyme preparations, in 200 mM-citrate, complete reversal of copper inhibition was not observed in the present work. With 4-nitrophenyl phosphate as substrate and with citrate as buffer, the inhibitory effect of 1 mM- Cu^{2+} ions was definite with both seminal and prostatic enzymes of human origin.

Calcium ions (100 mM) were without effect on the cock enzyme in both buffers; on the acid phosphatases of human origin no inhibitory effect was found, indeed a slight apparent potentiation (~10%) was always observed. Using phenyl phosphate as substrate and Ca^{2+} ions (10 mM) in acetate buffer (pH 5), Abul-Fadl & King (1949) observed an inhibition of about 50% on both human seminal and prostatic acid-phosphatase activities.

We do not, at the present time, feel in a position to comment on these discrepancies between our findings and those of Abul-Fadl & King (1949) and of Tsuboi & Hudson (1955) as our interests are primarily concerned with avian metabolism and not with that of the mammal.

Possible functions of acid phosphatase in the seminal plasma of the cock are puzzling. The concentration of phosphorylated organic compounds in the plasma which might act as substrates is very low (P. E. Lake, unpublished observations) and in any case it is doubtful whether the enzymes would be active at the neutral or slightly acid pH of the seminal plasma. Indeed, its presence in the seminal plasma may be purely adventitious. It remains to be investigated whether it has any influence on the activity of the spermatozoa during their sojourn in the vas deferens. Whether the apparent seasonal drop in activity, which was observed in the 30 cocks examined (Table 1), reflects a change in the androgen-oestrogen balance remains to be seen. In man, King & Delory (1945) found that plasma acid phosphatase rose with administration of androgens and fell with that of oestrogens.

SUMMARY

1. The seminal plasma of the domestic cock has been shown to contain a very active acid phosphatase(s); the enzyme is especially active against 4-nitrophenyl phosphate, and less so against the 2- and 1-phosphate of glycerol. It shows two broad activity peaks about pH 4.9 and pH 5.2.

2. An alkaline phosphatase is also present; its activity is considerably lower than (less than one-hundredth of) that of the acid enzyme and is of the same order as found in the blood plasma.

3. High acid-phosphatase activity (13 000 units) is shown by the secretions of the vas deferens. The activity of the fluids from the erectile cloacal tissues is relatively weak (1620–5800 units).

4. The nature of the acid phosphatase differs from that present in human seminal plasma or prostate as it is not inhibited by dextrorotatory tartrate. Its behaviour towards Cu^{2+} , Ca^{2+} and F^{-} ions is similar.

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Biochem. J. (1962) **82**, 281

Inactivation of Anaerobic Glycolysis in Fractions of Rat-Brain Homogenates

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(Received 26 July 1961)

It has been shown that under anaerobic conditions the rate of production of lactic acid from glucose by a brain supernatant fraction is markedly stimulated by the addition of brain mitochondria, although the mitochondrial fraction itself has quite a low glycolytic activity (Rossiter, 1957; Abood, Brunngraber & Taylor, 1959; Cremer, 1960; Hochstein & Cohen, 1960; Johnson, 1960). Stimulation is here defined as the excess of activity of a mixture of mitochondrial and supernatant fractions over the summed activities of the separate components. A reasonable explanation is that a relative deficiency of hexokinase in the supernatant is made good by the addition of the mitochondria with which most of rat-brain hexokinase is associated (Johnson, 1960). If fructose 1,6-diphosphate were the substrate instead of glucose in a medium containing an adequate adenosine diphosphate-regenerating system, the

lactate production by a mixture of mitochondrial and supernatant fractions would be expected to represent the summed activities of the mixture components with little or no stimulation, since all the enzymes involved are distributed similarly among the fractions of a rat-brain homogenate [75–90% of each being present in a crude supernatant fraction (Johnson, 1960)]. However, Brunngraber & Abood (1960) have reported that a large stimulation is obtained when a brain mitochondrial fraction is mixed with supernatant and assayed in a medium containing fructose 1,6-diphosphate and an adenosine diphosphate-regenerating system. Under the same conditions glycolysis of both the supernatant fraction and the mixture, but not the mitochondria, was stimulated by addition of purified muscle aldolase. They concluded that aldolase was rate-limiting in the supernatant and in the mixture, and that a dis-