the colorimetric method given by BERGMEYER[5]. The experimental tubes contained 0.25 mg of γ-globulins (Nutritional Biochemicals Corporation, Cleveland, Ohio), bovine albumins (L. Light and Co. Ltd., Colnbrook, England), glutamic acid (B.D.H.) and lysine (B.D.H.).

The enzyme activity was higher in the homogenates of kidney than in the liver. In general, the kidney enzyme activity was elevated by the γ-globulins, albumins and glutamic acid and inhibited by lysine. But the elevation by the γ-globulins and the inhibition by lysine were not statistically significant. Albumins and glutamic acid significantly elevated the enzyme activity. The liver enzyme activity was elevated by all the proteins and amino acids added, the elevation by the γ-globulins and lysine was significant, whereas the effect of albumins and glutamic acid was non-significant. The results show that the regulation of GPT is different in both the tissues. In other words, the relatively negative charge density contributed by the albumins and glutamic acid favours the kidney enzyme, while it has no action on the liver enzyme. Conversely, the relatively positive charge density in the homogenate contributed by the γ-globulins and lysine accelerates the liver enzyme, while it has no effect on the kidney enzyme. It is therefore suggested that the differential regulation of the same enzyme by different charges might be due to organ-specificity[6].

Glutamate pyruvate transaminase activity in the sucrose homogenates of the amphibian kidney and liver in relation to protein and amino acid charges

Tissue	No. of observations	Control	γ-globulins	Albumins	Glutamic acid	Lysine
Kidney	6	78.00 ± 10.05	82.00 ± 9.13 NS	98.00 ± 13.05 S	92.00 ± 8.16 S	69.00 ± 10.21 NS
Liver	5	27.00 ± 4.00	45.00 ± 10.81 S	30.00 ± 7.45 NS	33.00 ± 8.89 NS	42.00 ± 12.21 S

Activity is expressed as GPT units/mg protein. S, values are significant; NS, values are non-significant.

Zusammenfassung. Die Wirkung der Protein- und Aminosäurebelastung auf die Glutamat-Pyruvat-Transaminase in Niere und Leber von *Rana hexadactyla* wurde untersucht. Die Enzymaktivität war in der Niere höher als in der Leber. γ-Globulin und Lysin erhöhte die Leberenzyme und war ohne Einfluss auf die Nieren-Transaminase.

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The Binding of Cardiac Glycosides to Bull Spermatozoa

Investigations of net changes in alkali cation content[1-3] and of Na^+-K^+-activated ATPase activity[4,5] have provided some evidence for active transport of Na^+ and K^+ in mammalian spermatozoa. Recently the technique of specifically binding ^3H-labelled inhibitors of cation transport (ouabain and digoxin) has been used to demonstrate active transport sites on cell membranes and to estimate the number of such sites[6-9]. The present report describes the application of this technique to bull spermatozoa.

Materials and methods. Semen samples collected from 2 normal Jersey bulls were cooled slowly to 20 °C, and spermatozoa separated from seminal plasma by centrifugation (1200 *g*, 10 min at 20 °C). The spermatozoa were washed once and resuspended in an artificial bull seminal plasma (ABSP)[3]. Washed cells were inspected microscopically for motility, then counted and monitored electronically[10].

Naturally-decapitate spermatozoa from a Guernsey bull were separated into fractions of heads and motile midpiece-tails by rate-sedimentation on an ABSP:sucrose density gradient in a zonal centrifuge rotor (A type, M.S.E. Ltd.).

Cell concentration was adjusted to 5×10^8 cells/ml and cells preincubated at 37 °C for 15 min in ABSP. Tritiated digoxin or ouabain was then added usually to a final concentration of $5 \times 10^{-6} M$. Following a further 30 min incubation, the binding reaction was stopped by the addition of a large excess of $10^{-3} M$ ouabain in ABSP, and the cells washed 3 times in 50 vol. of ABSP by centrifugation (2000 *g*, 10 min). The final pellet was dissolved in 2 ml NCS solubilizer (Nuclear-Chicago), scintillator added, and counted in a Packard Tricarb Spectrometer. Labelled ouabain was obtained from New England Nuclear Corp. and digoxin from Dr. A. RUTSCHMANN, Sandoz AG, Basle (Switzerland).

Results and discussion. The time-course of binding at an ouabain concentration of $5 \times 10^{-6} M$ was determined, from which the reaction appeared to be complete after 15 min. The concentration of glycoside chosen in these experiments, $5 \times 10^{-6} M$, represents a concentration high enough to saturate most of the transport sites. Using ^3H-digoxin instead of ^3H-ouabain nearly equivalent numbers of bound molecules per cell were obtained. The glycoside bound to the spermatozoa was not removed to any signifi-

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cant extent by washing the cells an extra 2 or 3 times, indicative of an irreversible binding. The 3 washes routinely used eliminated the possibility of any free labelled glycoside remaining in the pellet.

For specific binding of cardiac glycoside to ATPase preparations, there is a requirement for Mg^{2+}, ATP or nucleotide triphosphate or certain other compounds to be present[6]. Applied to intact cells, metabolic inhibitors should decrease the internal ATP level, with a consequent decrease in glycoside binding. Table I shows significant effects of pre-treatment with a range of metabolic inhibitors, on the amount of bound digoxin or ouabain. Under the experimental conditions bull spermatozoa produce most of their ATP by fructolysis[11]. It is therefore probably significant that glycolytic inhibitors produced the greatest reduction in binding, cyanide having a relatively smaller effect. Fluoride and iodoacetate are known to have direct effects on membrane-ATPase transport systems, and may here be affecting binding directly rather than by a reduction in cellular ATP levels. Iodoacetamide, however, is known to inhibit glycolysis strongly at concentrations in the range 1–5 mM, without any inhibitory effect on the red cell sodium transport system[12] and must in this case be exerting its primary effect on the glycolytic system.

Table I. The effect of metabolic inhibitors on glycoside binding in bull spermatozoa

Inhibitor	Glycoside	Bound glycoside 10^3 molecules/cell	
		Control	Treated
NaCN (5 mM)	Digoxin	6.9	4.9
NaCN (5 mM)	Digoxin	7.3	5.4
NaF (20 mM)	Digoxin	4.4	0.8
NaF (20 mM)	Digoxin	4.3	2.1
Iodoacetate (1 mM)	Digoxin	5.6	1.3
Iodoacetate (1 mM)	Ouabain	6.7	3.6
Iodoacetamide (1 mM)	Digoxin	6.7	1.7
Iodoacetamide (1 mM)	Ouabain	6.7	3.1

Washed bull spermatozoa were preincubated with ABSP and inhibitor for 15 min at 37 °C. ^{3}H-ouabain or ^{3}H-digoxin was added to a final concentration of $5 \times 10^{-6} M$, and after a further incubation for 30 min the cells were washed by centrifugation and counted by liquid scintillation.

Table II. The effect of detergents on ouabain binding in bull spermatozoa

Treatment	Bound ouabain 10^3 molecules/cell
Control	4.24
Pretreated with Triton X-100	0.55
Pretreated with Saponin	0.33
Triton X-100 after ouabain binding	0.89
Saponin after ouabain binding	2.41

Spermatozoa were incubated for 5 min in ABSP containing either 0.02% Saponin or 0.1% Triton X-100 at 20 °C. After washing, the cells were incubated with $5 \times 10^{-6} M$ ^{3}H-ouabain at 37 °C for 30 min. Cells previously exposed to ^{3}H-ouabain for 30 min, were also treated with Saponin or Triton X-100 for 5 min, washed and processed for counting.

Table III. Ouabain binding in separated heads and midpiece-tails of Guernsey bull spermatozoa

	Bound ouabain 10^3 molecules/particle	
	Experiment 1	Experiment 2
Heads	0.36	0.71
	0.31	0.35
Heads + 1 mM iodoacetamide	–	0.46
	–	0.48
Midpiece-tails	2.0	3.2
Midpiece-tails + 1 mM iodoacetamide	1.5	2.8
	1.7	2.6

Heads and midpiece-tails were fractionated by zonal centrifugation. After recovery from the gradient material cells were washed and incubated with ^{3}H-ouabain, $5 \times 10^{-6} M$ for 30 min at 37 °C.

Table III indicates that binding of ouabain mostly occurs in the midpiece-tail fraction of fractioned spermatozoa, and appears to be sensitive to metabolic inhibitors. The sum of binding in the separated components appears to be very similar to the binding in intact spermatozoa, although the sensitivity of isolated cell components to glycolytic inhibitors of binding was significantly less than that of intact cells. The evidence presented suggests that the glycoside-binding sites and hence the $Na^+ + K^+$ activated ATPase and cation transport system are localized on the outer cell membrane of the midpiece-tail region. At least 70% of the total glycoside bound in 30 min at a concentration of $5 \times 10^{-6} M$ was sensitive to glycolytic inhibitors or detergents, and thus the number of specific binding sites on intact bull spermatozoa was in the range $3–6 \times 10^3$ per cell. This figure may be compared with the range of values reported for other tissues, i.e. 6 for LK sheep erythrocytes[15] and $1–5 \times 10^5$ for a variety of mammalian cultured cells[16,17].

Resumen. Se ha investigado la unión de glicósidos cardiacos al espermatozoide de toro. Dicha unión es sensible a inhibidores metabólicos y fué localizada en la membrana celular del segmento intermediario y la cola.

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