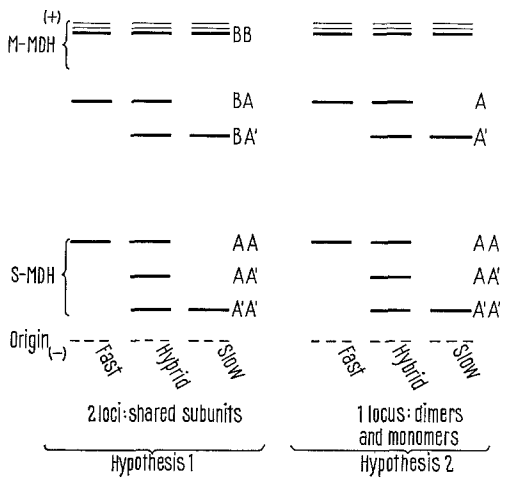

Fig. 2. Subunit composition of variant supernatant malate dehydrogenase (S-MDH) isozymes. Left: Hypothesis 1 a) both A and B subunits are encoded in duplicate S-MDH loci. The BB S-MDH isozyme coincides in electrophoretic mobility with the M-MDH isozyme or b) B subunits are encoded in the M-MDH locus and A subunits are encoded in the S-MDH locus. The appearance of BA or BA' isozymes represent sharing of subunits from different loci. Right: Hypothesis 2. The S-MDH may exist as both dimers and monomers (with the dimers possessing slower mobility because of sieving action of the gel).

phenotype (Figure 1, right) was only observed in one fish which was probably heterozygous at *both* the S-MDH locus and the M-MDH locus.

In conclusion, *F. heteroclitus* has codominant allelic variants at both the S-MDH locus and the M-MDH locus. The genetic variants encoded in both loci are evenly distributed among males and females which suggests that they are autosomally inherited. The high degree of isozyme polymorphism observed for malate dehydrogenase has also been observed for other *Fundulus* isozymes, e.g., lactate dehydrogenase[3,14] and esterases[15]. Thus, *F. heteroclitus* appears to be an excellent organism to employ for investigating the linkage relationships of genes encoding these isozymes[16].

G. S. WHITT[17]

Department of Biology, Yale University, New Haven (Connecticut 06520, USA), 21 January 1970.

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[17] Present address: Department of Zoology, University of Illinois, Urbana (Illinois 61801, USA).

Active Transport in the Rabbit Blastocyst

The rabbit embryo begins to accumulate fluid to form a central cavity between 3 and 4 days after mating. Initially this process is slow but the efficiency of transport increases with time so that by the ninth day each cell transports about 20 times its own volume of fluid every hour[1]. During the earlier stages of development, fluid within the blastocyst contains high concentrations of potassium and bicarbonate and low concentrations of sodium and chloride relative to serum, but later the concentrations of all 4 ions approach those found in serum[2–4]. The present work uses cultured blastocysts to study some of the factors controlling water and ion movements across this tissue.

New Zealand white rabbits weighing 2–3 kg were killed by the i.v. injection of pentobarbiton sodium, exactly 6 days after mating. The uterus was removed and the blastocysts exposed by blunt dissection. Each blastocyst was placed on a platinum loop to be weighed on a sensitive torsion balance (0–50 $\pm$ 0.1 mg) before being transferred to 1 ml tissue culture medium '199' (Glaxo Labs. Ltd., Greenford, England) containing an additional 12 m*M* NaCl. The culture medium was maintained at 37 °C and gassed with 95% air + 5% CO_2. Blastocysts were later removed, weighed and samples taken for analysis. To analyze for bicarbonate the blastocyst was punctured and 10–20 µl fluid sucked immediately into a Natelson microgasometer. The sample was sealed with mercury, its volume read and the bicarbonate content then determined in the usual way. Other samples were analyzed for sodium and potassium by flame photometry (Evans Electro Selenium EEL) after suitable dilution with distilled water. Chloride determinations were carried out by micro-titration against silver nitrate using a potentiometric method to detect the end point[5]. Particular care was taken to obtain and dilute samples as soon as possible after removing blastocysts from the culture medium to avoid errors caused by evaporation.

The blastocyst being a closed system must produce cells by mitosis to provide space for transported fluid. The contribution new cells make to total fluid transport can be allowed for by assuming that both the cell size and water influx per unit surface area remain constant during culture. Then the total influx into a spherical blastocyst of radius r is $4\pi r^2 f$, where f is the constant influx per unit surface area. The radius increases at a constant rate and the time needed for the blastocyst to double its volume (the doubling time) can be calculated as $0.26/f \cdot r_0$, where r_0 is the initial radius. It was decided to use the doubling time as a measure of the rate of fluid transport. This time did depend however on r_0 and therefore on the initial size of the blastocyst. For blastocysts weighing from 5–25 mg, taken 6 days after mating and cultured for 7 h, the doubling time varied from 8.2 to 12.2 h. The correlation between the initial weight and doubling time was significant ($r = 0.56$, $P < 0.05$, $t = 2.8$, 19 observations). Errors from this source were minimized by ensuring that blastocysts chosen for each experimental series represented the whole range of initial weights.

Figure 1 shows the time course of blastocyst expansion in culture medium over an 8-h period. The calculated doubling time rose from 4 h, for a 1-h incubation, to 10 h

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after incubation for 8 h, most of this increase occurring during the first 3 h of incubation. Some fluid movement in the early stages might result from a slight osmotic imbalance between the culture medium and the fluid within the blastocyst. A culture time of 7 h was chosen for subsequent experiments. By this time the blastocysts had increased their volume by 70%, osmotic effects had become negligible in relation to total transport and the doubling time was found to be reasonably constant (10.3 ± 0.46 h, mean ± S.E.).

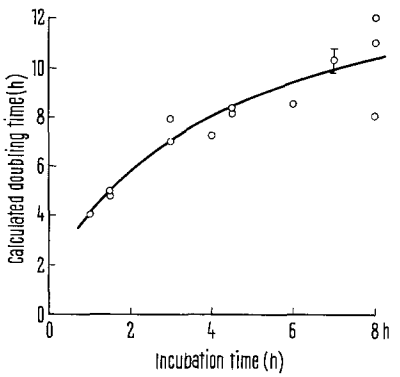

Fig. 1. Time course for blastocyst expansion in tissue culture medium. Blastocysts, removed from rabbits 6 days after mating, were placed in culture medium at 37 °C, gased with 95% air + 5% CO_2. The difference between the initial and final weight was used to calculate the time needed for the blastocyst to double its volume. Points represent single observations with the exception of that determined after 7 h incubation which gives the mean ± S.E. of 19 observations.

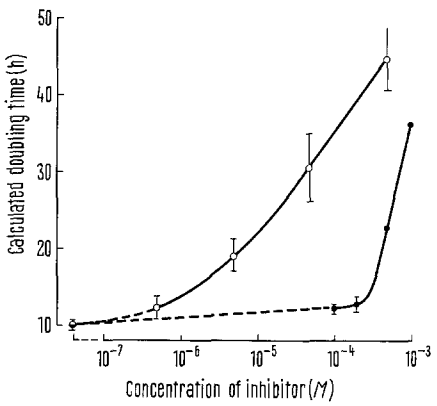

Fig. 2. Effect of ouabain and acetazolamide on fluid transport in the rabbit blastocyst. Blastocysts were weighed and incubated in tissue culture medium for a period of 7 h as described in Figure 1. Each point gives the mean of from 5–19 observations ± S.E. ○—○, ouabain; ●—●, acetazolamide.

Figure 2 shows the effect 2 inhibitors of ion transport, ouabain and acetazolamide, had on the calculated doubling time measured after a 7-h incubation. The log concentration-effect curve was nearly linear over a hundred-fold range of concentration for ouabain but with acetazolamide the change from no inhibition to full inhibition took place very suddenly. Concentrations of ouabain ($5 \times 10^{-4} M$) and acetazolamide ($10^{-3} M$) were chosen to block fluid transport in further experiments to determine their effects on the transport of different ions.

The Table shows the mean concentrations of Na^+, K^+, Cl^- and HCO_3^- found in blastocyst fluid taken immediately after removal from the uterus or after incubation for 7 h in culture medium. The initial concentrations of all 4 ions were similar to values reported previously[3,4]. A large net transfer of Na^+ and Cl^- accompanied water transport into the blastocyst incubated under control conditions but there was no net transport of K^+ or HCO_3^-. The concentrations of these latter 2 ions were about halved during incubation due to the influx of water. Cooling the blastocyst reduced net movements of Na^+ and Cl^-, part of the Cl^- influx now appearing to be in exchange for HCO_3^-. There was no net transport of K^+ and the residual flux of water was probably caused by the 2 solutions coming into osmotic equilibrium. Acetazolamide inhibited water transport and caused a much greater loss of HCO_3^- and gain in Cl^- than did cooling. Blastocysts incubated with acetazolamide also lost K^+. The reduction in Na^+ transport was not complete. Ouabain inhibited the water flux to an extent equal to cooling or treatment with acetazolamide and there was some exchange of Cl^- for HCO_3^-, though less than when acetazolamide was used. The major effect of ouabain was to inhibit the transport of both Na^+ and Cl^-.

Ouabain inhibits transport ATPase and its effect on Na^+ transport in the blastocyst suggests that this enzyme pumps sodium out of the trophoblast cells into the blastocoel during development. The action of acetazolamide is more obscure, it inhibits carbonic anhydrase[6], an enzyme known to be present in rabbit blastocysts[7], but it also

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Ionic composition of rabbit blastocyst fluid taken 6 days after mating, before and after incubation in tissue culture medium

Conditions of incubation	Concentration (mM)				Net transport (μmoles)				Water (μl)
	Na^+	K^+	Cl^-	HCO_3^-	Na^+	K^+	Cl^-	HCO_3^-	
Culture medium	135	5.0	138	7					
Time 0	144	9.2	88	71					
7 h/37 °C	128	5.7	103	33	7.0	0	8.8	0.1	68.2
7 h/0 °C	134	9.3	94	59	0.5	0.1	1.7	—0.6	18.2
Acetazolamide, 7 h/37 °C/$10^{-3} M$	144	5.1	118	32	2.5	—0.3	5.5	—3.7	18.1
Ouabain, 7 h/37 °C/$5 \times 10^{-4} M$	140	7.2	99	45	2.1	—0.1	2.8	—1.7	18.6

The ionic composition of the culture medium is also given for comparison. The net transport of different ions and water have been calculated assuming 100 μl of blastocyst fluid, of the composition given as time 0 in the Table, to be present at the start of incubation. Each value gives the mean of from 4–11 analyses. Analyses for Na^+, K^+ and Cl^- were performed on identical samples; HCO_3^- determinations were made on separate samples.

inhibits active chloride transport in tissues such as the gastric mucosa[8] and the relationship between these two effects is far from clear. Neither are the effects of ouabain and acetazolamide completely specific for single transport processes, an overlap occurs similar to that seen in kidney tubular absorption where ouabain or removal of HCO_3^- both cause almost complete inhibition of Na^+ reabsorption[9]. Both transport ATPase and carbonic anhydrase appear essential for optimal transport to occur, but the exact nature of the interdependence between the two remains obscure[10].

Résumé. Des blastokystes ont été pris à des lapins 6 jours après l'accouplement et couvés in vitro pendant 7 h. Le transport d'eau et de chlorure de soude a été arrêté par l'ouabaine ou par refroidissement. L'acétazolamide met en équillibre les concentrations de bicarbonate-chlorure. Il nous semble donc que c'est le transport de l'ATPase qui extrait le chlorure de soude et que l'anhydrase carbonique y concourt maintenant une concentration elevée de bicarbonate à l'intérieur du blastokyste.

M. W. SMITH

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Induced Changes of Action Potential on Cardiac Contraction

According to current views[1-3], the action potential of mammalian ventricle initiates contraction (a) by causing Ca ions to enter the cell through the surface membrane and (b) by liberating calcium from intracellular stores. In the present experiments it has been shown that the level of membrane potential during the first third of an action potential determines the rate of tension development and sets the 'time to peak'. The present results are complementary to those previously reported by MORAD and TRAUTWEIN[1].

Methods. Sheep or calf right ventricular trabeculae were placed in a 3-compartment chamber as described by WOOD et al.[3]. The middle chamber was perfused with sucrose solution and the other 2 with Tyrode. Current was made to flow between the 2 ends of the bundle which were in contact with Tyrode solution. Tension and intracellular potential were recorded from a length of less than 1 mm. The driving rate was 24/min.

Results. At a temperature of 32 °C the action potential lasted for about 400 msec (Figure 1). Pulses of 50 msec decreased the rate of tension development (dP/dt) when the amplitude of the action potential was increased (Figure 1, A) and had the opposite effect when the amplitude of the action potential was decreased (Figure 1, B). These effects on tension were largest when the pulses were applied early during activity. After about 150 msec following the onset of the action potential, the pulses had no effect on either dP/dt or on the time to peak.

Pulses of several hundred msec, which increased the amplitude and the duration of the action potential (Figure 2), resulted in a decrease of the initial twitch; tension was partially maintained throughout the interval of membrane depolarization and upon switching off the d.c. pulse, concomitant with membrane repolarization,

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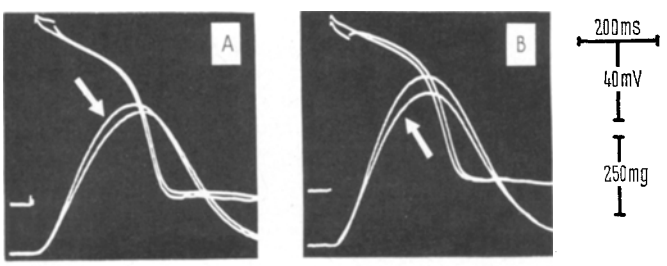

Fig. 1. Superimposed action potentials (upper records) and contractions (lower records) of a sheep ventricular bundle, 0.8 mm thick. Arrows point to the tension records as obtained under control conditions. Constant current (0.02 mA) made to flow for 50 msec, beginning 10 msec after the driving stimulus. The rate of tension development was depressed by increasing the action potential amplitude (A), and enhanced by decreasing the action potential amplitude (B).

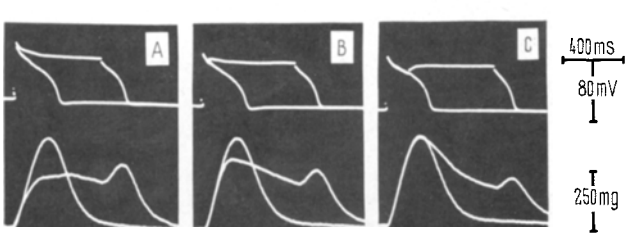

Fig. 2. Superimposed records from a calf ventricular bundle, 0.7 mm thick. A pulse of constant current (0.02 mA, 550 msec) was applied 10, 60 and 160 msec after the driving stimulus. Note the effect on the initial twitch, on maintained tension, and on the appearance of a second twitch.