LOW NA^+ CONCENTRATION: A FACTOR CONTRIBUTING TO DIMINISHED UPTAKE AND INCORPORATION OF AMINO ACIDS BY DIAPAUSING MOUSE BLASTOCYSTS? (1)

LON J. VAN WINKLE
Department of Natural Sciences, The University of Michigan-Dearborn, Dearborn, Michigan 48128

ABSTRACT Uptake and incorporation of ^{14}C amino acids by normal and diapausing blastocysts was found to be Na^+ dependent with one exception. Delayed embryos preincubated 25 h in medium containing 124 mM Na^+ had labeling characteristics independent of the Na^+ concentration present during labeling. Since diapausing embryos preincubated 25 h in low Na^+ medium (55 mM) retained their Na^+ dependent labeling characteristics, delayed blastocysts may be in a low Na^+ environment in situ. Low Na^+ may explain why delayed blastocysts are unable to utilize radioactive methionine in vivo (Weitlauf and Greenwald, '68).

Delayed blastocysts characteristically incorporate radioactive amino acids into protein more slowly than normal blastocysts both in vivo (Weitlauf and Greenwald, '68) and in vitro (Weitlauf, '73; Van Winkle et al., '72; Van Winkle, '75). One hypothesis is that an inhibitor(s), present in the uterus during diapause, contributes to nidation delay by slowing metabolic processes (Weitlauf, '74; Psychoyos, '73). A substance which inhibits incorporation of radioactive RNA precursors into these macromolecules in blastocysts in vitro has been detected in both rat (Psychoyos, '73) and mouse (Weitlauf, '76) uterine flushings during diapause. The paradox is that an inhibitory substance is also found in uterine flushings in situations associated in vivo with active embryos (Weitlauf, '76). Part of the explanation for this paradox may be that nidation delay involves multiple parameters, only one of which is an inhibitory substance. Washings from rat uteri during diapause contain only about half as

much Na^+ as washings from uteri during normal pregnancy (Setty et al., '73). Furthermore, it has been demonstrated that amino acid transport is Na^+ dependent in mouse blastocysts grown in vitro from the two cell stage (Borland and Tasca, '74). Since amino acid incorporation may depend, in part, on amino acid transport (Van Winkle, '75; Van Winkle and Dabich, '77) it is possible that low Na^+ in the delayed uterus may have contributed to the results observed for diapausing embryos both in vivo and in vitro. The observation that amino acid uptake and incorporation increases in delayed blastocysts upon in vitro incubation (Weitlauf, '73; Van Winkle, '75; Van Winkle et al., '77) might be explained just as effectively by Na^+ concentrations which are higher in vitro than in vivo as by the loss of an inhibitor produced by the uterus. The present studies were designed to test the hypotheses that: (a) amino acid uptake and incorporation by blastocysts from the uterus depend on the Na^+ concentration in the incubation medium and (b) the Na^+ concentration in the diapausing uterus is lower than in the usual in vitro incubation medium (Brinster, '71).

MATERIALS AND METHODS Swiss Webster mice were induced to ovulate and mate as described by Fowler and Edwards ('57). The day of vaginal plug detection was designated day 1 of pregnancy. Delay was induced via ovariectomy between 9:30 and 11:30 h on day 4 of pregnancy (Weitlauf and Greenwald, '68). Normal and diapausing blastocysts were obtained between 10:30 and 12:30 h on days 5 and 8 of pregnancy respectively.

High Na^+ medium contained almost as much Na^+ as Brinster's medium (BMOC-3; Brinster, '71) but had the following modifications: 68.9 mM NaCl, 29.9 mM sodium lactate, 11.1 mM glucose and 1.00 mg/ml bovine serum albumin (total osmolarity 280 mM, 124 mM Na^+). Low Na^+ medium was prepared by substituting choline chloride (Borland and Tasca, '74) for NaCl (total osmolarity 280 mM, 55 mM Na^+). (Blastocysts accidentally collapsed during manipulation were

TABLE 1

Effect of Na^+ concentration on total uptake and incorporation of ^{14}C amino acids by blastocysts just obtained from uteri

Type of Blastocyst	n	Na^+ concentration during labeling	cpm/embryo ± se	
			Total ^{14}C	^{14}C protein
Normal	19	Low	820 ± 89	305 ± 40
Normal	22	High	1501 ** ± 191	603 ** ± 67
Delayed	21	Low	320 ± 37	160 ± 15
Delayed	21	High	733 ** ± 65	378 ** ± 32

** $p < 0.01$

TABLE 2

Effect of Na^+ concentration on total uptake and incorporation of ^{14}C amino acids by blastocysts after 25 hours of in vitro preincubation

Type of Blastocyst	n	Na^+ concentration during 25 h preincubation	Na^+ concentration during labeling	cpm/embryo ± se	
				Total ^{14}C	^{14}C protein
Normal	17	Low	Low	960 ± 78	296 ± 15
Normal	16	Low	High	1425 ** ± 106	435 ** ± 24
Normal	18	High	Low	1181 ± 74	449 ± 26
Normal	17	High	High	1841 ** ± 111	529 * ± 27
Delayed	13	Low	Low	445 ± 51	146 ± 19
Delayed	11	Low	High	739 ** ± 77	188 * ± 12
Delayed	10	High	Low	1636 ± 128 n.s.	414 ± 41 n.s.
Delayed	12	High	High	1528 ± 166	404 ± 23

* $p < 0.50$
** $p < 0.01$

observed to re-expand in both high and low Na^+ medium.) All media were equilibrated at 37° C in an atmosphere of 5% CO_2 in air (100% humidity) before being used in experiments.

Blastocysts were flushed from excised uteri into the depression of a Maximov slide with a stream of high or low Na^+ medium (Brinster, '70). Embryos were collected and washed once in fresh medium. Blastocysts, fewer than 30 at a time, were then either preincubated 25 h or labeled immediately in 0.20 to 0.50 ml of the same medium used for flushing. A uniformly labeled ^{14}C amino acid mixture (4.4 μCi/ml medium, 54 mCi/matom carbon, CFB 104, Amersham Searle) was used for labeling. Blastocysts which had been preincubated in high or low Na^+ medium for 25 h were subsequently labeled in either high or low Na^+ medium. Moreover, some embryos which had been preincubated in high Na^+ medium were randomly selected and transferred to low Na^+ medium for 15 to 30 min (to simulate the time usually required to flush embryos from the uterus and to further manipulate them) before labeling. Similarly, some blastocysts preincubated 25 h in low Na^+ medium were labeled in high Na^+ medium.

Following two hours of incubation with radioactive amino acids, embryos were washed twice in the appropriate medium (>100:1 dilution per wash) then solubilized in 2% sodium dodecylsulfate ($\frac{W}{V}$). Each of the resultant solutions was quantitatively transferred to a Whatman 3MM chromatography paper suspended on a straight pin. After drying, 70 μl of 6% ($\frac{W}{V}$) trichloroacetic acid was added to the paper. The papers were dried, then placed in vials with scintillation fluid and counted in a Beckman LS8100 Scintillation Counter (maximum efficiency 96%). After counting, radioactive protein was determined by the method of Mans and Novelli ('61).

Data is reported as the mean cpm/embryo plus or minus the standard error (se) calculated from multiple determinations made in at least three separate

experiments. Means were compared statistically by group comparison tests (Woolf, '68). Each experiment was planned so that some determinations for each of the four categories in table 1 or the eight categories in table 2 were obtained on the same day from one group of normal and one group of delayed mice. Moreover, in one experiment determinations were made for all 12 categories in tables 1 and 2. Data from single experiments were consistent with the total data. Thus, trivial variables, such as different batches of media, mice, etc., were probably unimportant. Finally, procedural controls (samples with no embryos) were subtracted from experimental determinations before the above calculations.

RESULTS AND DISCUSSION Total uptake and incorporation of ^{14}C amino acids into protein by blastocysts of the same origin were higher in high Na^+ medium than in low Na^+ medium (tables 1 and 2). The same origin means here that embryos had recently been flushed from the same kind of uteri (either delayed or normal) (table 1) or had been preincubated together in the same medium for 25 h (table 2). The single exception to the preceding generalization was that total uptake and incorporation by delayed blastocysts preincubated 25 h in high Na^+ medium were independent of the Na^+ concentration in the medium during labeling (table 2). Thus, another effect of high Na^+ medium on diapausing blastocysts was to establish a relatively high but Na^+ independent state of amino acid uptake and incorporation. This Na^+ independent state may be quite stable, once established. In a preliminary experiment, delayed embryos incubated 50 h in high Na^+ medium, then 20 h in low Na^+ medium had labeling characteristics similar to diapausing embryos incubated 25 h in high Na^+. If high Na^+ leads to Na^+ independent uptake and incorporation of amino acids by delayed blastocysts in vivo then these embryos may be in a low Na^+ environment in situ. Low Na^+ could partially explain the inability of diapausing blasto-

cysts to utilize radioactive methionine for protein synthesis in vivo (Weitlauf and Greenwald, '68).

In contrast to diapausing embryos, normal blastocysts do not attain a Na^+ independent state after 25 h of in vitro incubation in high Na^+ medium (table 2). Thus, although delayed blastocysts come to resemble the more metabolically active, normal blastocyst upon in vitro incubation in high Na^+ medium (Weitlauf, '74, '76; Van Winkle, '75), they are not the same even after 25 h. If diapausing embryos are metabolically dormant (McLaren, '73; Weitlauf, '74, '76), their metabolic level apparently can be brought to normal by increasing the Na^+ concentration of the medium. Under some conditions the rate of utilization of amino acids and glucosamine is the same in normal and diapausing blastocysts (Van Winkle et al., '77). Moreover, a lower Na^+ concentration in delayed uterine fluid could lead to diminished uptake and incorporation of ^{14}C amino acids by diapausing embryos, but neither the rate of protein synthesis nor metabolism need be slower than in normal blastocysts. Attempts are presently being made to measure the Na^+ concentration in mouse uterine fluids.

ACKNOWLEDGMENTS The author wishes to thank Mr. Roger Acey, Dr. Bayne Carew, Ms. Mikki Holloway and Dr. Richard Norman for critically reading the manuscript and Ms. Chalina Hillson for typing it.

LITERATURE CITED

Borland, R. M., and R. J. Tasca 1974 Activation of a Na^+ dependent amino acid transport system in preimplantation mouse embryos. Develop. Biol., 36: 169-183.

Brinster, R. L. 1970 In vitro cultivation of mammalian ova. Advances in the Biosciences, 4: 199-233.

Brinster, R. L. 1971 In vitro culture of the embryo. In: Pathways to Conception. Charles G. Thomas Publishing Co., Springfield, Illinois.

Fowler, R. E., and R. G. Edwards 1957 Induction of superovulation and pregnancy in mature mice by gonadotropins. J. Endocrin., 15: 374-384.

Mans, R. J., and G. D. Novelli 1961 Measurement of the incorporation of

radioactive amino acids into protein by a filter-paper disc method. Arch. Biochem. Biophys., 94: 48-53.
McLaren, A. 1973 Blastocyst activation. In: The Regulation of Mammalian Reproduction. Charles G. Thomas Publishing Co., Springfield, Illinois, pp. 321-334.
Psychoyos, A. 1973 Hormonal control of ovaimplantation. Vitamins and Hormones. Advances in Research and Application, 31: 201-256.
Setty, B. S., Man Mohan Singh, S. R. Chowdhury and Amiya B. Kar 1973 The role of electrolytes of the endometrium and uterine fluid during delayed implantation in rats. J. Endocr., 59: 461-464.
Van Winkle, L. J. 1975 Factors affecting net incorporation of radioactive amino acids into protein of normal and diapausing mouse blastocysts. Ph.D. dissertation, Wayne State University, Detroit.
Van Winkle, L. J., R. A. Acey and D. Dabich 1977 Contrasts between ^{14}C-amino acid and ^{14}C-glucosamine uptake and incorporation in early diapausing mouse blastocysts. Abstracts of the Fifth International Conference on Birth Defects, Excerpta Medica Foundation, 305 Keizersgracht, Amsterdam.
Van Winkle, L. J., D. Dabich and T. J. Andary 1972 Amino acid incorporation into normal and delayed implantation blastocysts. J. Cell Biol., 55: 266a.
Van Winkle, L. J., and D. Dabich 1977 Transport of naturally occurring amino acids and alpha amino isobutyric acid by normal and diapausing mouse blastocysts. Accepted. Biochem. Biophys. Res. Comm.
Weitlauf, H. M. 1973 In vitro uptake and incorporation of amino acids by blastocysts from intact and ovariectomized mice. J. Exp. Zool., 183: 303-308.
Weitlauf, H. M. 1974 Metabolic changes in the blastocysts of mice and rats during delayed implantation. J. Reprod. Fert., 39: 213-224.
Weitlauf, H. M. 1976 Effect of uterine flushings on RNA synthesis by implanting and delayed implanting mouse blastocysts in vitro. Biol. of Reprod., 14: 566-571.
Weitlauf, H. M., and G. S. Greenwald 1968 Influences of estrogen and progesterone on the incorporation of ^{35}S methionine by blastocysts in ovariectomized mice. J. Exp. Zool., 169: 463-470.
Woolf, C. M. 1968 Principles of Biometry, First Edition. D. Van Nostrand Company, London: 62-67.

REFERENCES

1 This study was supported by The University of Michigan-Dearborn Faculty Research Grant 238592.