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COMPLETE DEVELOPMENT *IN VITRO* OF THE PRE-IMPLANTATION STAGES OF THE MOUSE IN A SIMPLE CHEMICALLY DEFINED MEDIUM

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Whitten (1956) showed that 8-cell mouse embryos develop into blastocysts in a simple chemically defined medium containing glucose, and McLaren & Biggers (1958) demonstrated that blastocysts cultured in this way produce normal mice when transferred into uteri of foster mothers. Whitten (1957) also showed that late 2-cell mouse embryos developed into blastocysts if lactate was incorporated in the medium but earlier stages did not cleave under these conditions. Thus the observation that mouse zygotes develop into normal blastocysts in the lumen of oviducts in organ cultures (Biggers, Gwatkin & Brinster, 1962) suggested that special conditions for initial development are provided by the tube. Recently, Whittingham & Biggers (1967) demonstrated that 1-cell embryos cleave to the 2-cell stage in a simple medium containing lactate and pyruvate (Brinster, 1965), and that these 2-cell stages develop into blastocysts in organ cultures of the ampullary region of the Fallopian tube. The unknown requirements provided by the Fallopian tube appeared to operate only in the intermitotic period between the first and second cleavage. In this paper we describe a simple chemically defined medium in which the eggs of certain hybrid mice develop from the 1-cell stage to the blastocyst completely *in vitro*. These blastocysts are capable of implantation and thus we have overcome the final barrier which prevented complete development *in vitro* of the pre-implantation stages of a mammal independent of the female genital tract.

Eggs were obtained from F_1, C57BL/10J × SJL/J females on the morning on which a copulation plug was observed after pairing with a BALB/cDg or F_1 BALB/cDg × 129/J male. The cumulus was completely removed by hyaluronidase in Dulbecco's phosphate-buffered saline (pH 7·2, 440 U.S.P. units/ml), and the eggs were washed twice with the culture medium, inspected for pronuclei and polar bodies, and then cultured in 1 ml of medium in 5-ml plastic tubes using the method described by Biggers, Whitten & Whittingham (1968). The composition of the medium is presented in Table 1.

One hundred and sixty-three 1-cell eggs from the hybrids were cultured in this manner and 122 apparently normal blastocysts, many of which were expanded, developed at the normal rate. Five viable offspring were obtained from eighteen of these blastocysts after they were transferred to uteri of foster mothers. In contrast, blastocysts were observed less frequently when eggs from

other strains of mice were cultured (Table 2). In the case of the random bred oocytes, late 2-cell embryos cultured simultaneously with the 1-cell embryos readily developed to blastocysts while the 1-cell embryos did not.

The observation that the zygotes of this hybrid mouse will develop to normal blastocysts in a chemically defined medium shows that the block to development in the intermitotic period between first and second cleavage observed previously (Whittingham & Biggers, 1967) is not due to the lack of a specific molecule secreted by the Fallopian tube which triggers this stage of development. In fact the combined evidence from the culture of mouse embryos *in vitro* in chemically defined media now shows that early development is not regulated by specific

TABLE 1

COMPOSITION OF THE MEDIUM FOR THE CULTURE OF 1-CELL MOUSE OVA

Component	*g/l*	*mM*
NaCl	4·000	68·49
KCl	0·356	4·78
L(+) Ca lactate $4H_2O$	0·496	1·71
KH_2PO_4	0·162	1·19
$MgSO_4 . 7H_2O$	0·294	1·19
$NaHCO_3$	2·106	25·07
Na pyruvate	0·036	0·33
Na lactate (3·68 ml/l of syrup)	2·416	21·58
Glucose	1·000	5·56
Crystalline bovine albumin	4·000	
Streptomycin sulphate	0·050	
Penicillin, potassium salt	0·075	
Distilled water to 1000 ml		

TABLE 2

DEVELOPMENT *in vitro* OF ZYGOTES FROM DIFFERENT STRAINS OF MICE

Strain of mice	*No. of zygotes cultured*	*No. of blastocysts observed*
F_1 C57BL/10J × SJL/J	163	122
SJL/J	97	3
C57BL/10J	11	2
129/Rr	18	3
Random bred (1-cell) } Cultured	50	0
Random bred (2-cell) } simultaneously	77	60

macromolecules of maternal origin. Nevertheless it would be wrong to conclude that the secretions of the Fallopian tube do not provide unique conditions for cleavage. A considerable body of evidence shows that the cleaving ovum requires specific exogenous energy sources at different times of development (Biggers, Whittingham & Donahue, 1967), and these may be supplied by secretions of the Fallopian tubes.

Why the medium shown in Table 1 allows development through the first two cleavage divisions is not yet clear since several changes have been made from

the earlier formulations. However, the following points are worth noting. The new medium is more dilute and has an observed osmolarity of 242 m-osmols, while the calculated value, assuming complete ionization and no interactions, is 256 m-osmols compared with 308 m-osmols for the media in general use (Brinster, 1965). The decrease is entirely due to a reduction in the content of sodium chloride. Also, the concentration of crystalline bovine plasma albumin has been increased from 1 to 4 g/l. The relative importance of these factors is being investigated.

The ready development of the zygotes from the hybrid may have a genetic explanation. These zygotes are derived from the fertilization of an F_1 hybrid oocyte and their development may be an expression of heterosis in the oocyte which is manifest biochemically. There is evidence from studies on the maturation of mouse oocytes in chemically defined media that the restricted pattern of energy metabolism of the cleavage stages is determined during oogenesis (Biggers *et al.*, 1967). It is also known that the intermitotic period between first and second cleavage is associated with profound biochemical changes, such as the ability to use incorporated lactate to support cleavage (Wales & Whittingham, 1967), and to deposit glycogen rapidly (Stern & Biggers, 1968). An alternative explanation is that essential metabolites are less readily leached *in vitro* from the hybrid oocytes.

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