proteins for acetaldehyde. As shown in the figure this procedure allows the determination of acetaldehyde in plasma in a range from 1 to 200 μM.

On the basis of these results the following procedure was adopted to determine acetaldehyde in human blood. 2 ml of blood were collected from the cubital vein with a butterfly needle with 3 inches of tubing, allowing the blood to drop into a graduated 5-ml centrifuge tube containing 2 ml 100 mM chloralhydrate in 0.9% NaCl with 100 U-USP heparin. After mixing the plasma was isolated in the covered tube by centrifugation. 2 ml of the plasma were transferred into glass vials (total volume of 26 ml) containing 0.1 ml 60% perchloric acid and 1.0 ml internal standard solution containing 0.1 mg 1-propanol. The vial was immediately sealed with a rubber membrane and equilibrated at 60 °C for 30 min in a water bath. 5 ml of the gas phase were injected into a Perkin-Elmer 900 gas chromatograph equipped with a flame ionization detector and an Autolab Minigrator from Spectra-Physics. A glass column (1.8 m × 2 mm inner diameter) packed with Porapak Q 80-100 mesh was used. The operating conditions were: injector temperature 180 °C; column temperature 150 °C; detector temperature 290 °C; carrier gas: 30 ml/min of nitrogen. The retention times for acetaldehyde, ethanol and 1-propanol were 97, 151 and 383 sec. Samples of precipitated plasma in the sealed vials were stable at room temperature for 24 h.

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The collagen substratum influences in vitro hatching and attachment of the mouse blastocyst in a serumless medium

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Summary. When a serumless medium is used for the in vitro growth and development of post-blastocyst mouse embryos, a collagen substratum causes a delay in the hatching from the zona pellucida. However, the collagen substratum is essential for blastocyst attachment and trophoblast cell outgrowth after hatching has taken place.

The in vitro growth and development of the post-blastocyst mouse embryo to the egg cylinder stage has been described previously[3-6]. The influence of culture media[7,8], amino acids[9], glucose[10,11], and nucleosides[12] on this stage of mammalian differentiation has been studied. All of these studies have utilized a serum component as part of the culture medium. Although information is available concerning the influence of a collagen substratum on hatching and attachment in serum supplemented media[13], little information is available utilizing a serumless medium. The objective of this study was to compare a collagen to a plastic substratum with respect to the in vitro hatching and attachment of the mouse blastocyst in a serumless medium.

Superovulation was induced in random bred Swiss mice by the method of Gates[14]. Injected female mice were caged overnight with male mice and mating was verified the following morning by the presence of a copulatory plug in the vagina. Ovulation was assumed to occur approximately 12 h after the HCG injection[15]. 4 days postmating blastocysts were recovered by flushing the excised uterus with 0.5 ml of modified Brinster's medium[16]. Recovered blastocysts were pooled in culture medium under silicone oil in an atmosphere of 95% air plus 5% CO_2. Previously sterile 60 × 30 mm plastic petri dishes were either layered with collagen reconstituted from rat tails as described by Hsu et al.[17], or used without the collagen layer. Individual petri dishes were equilibrated with 5 ml of the culture medium 60 min prior to the introduction of the mouse blastocysts. Previously pooled blastocysts were randomly assigned to either collagen or plastic substratum with Eagle's basal medium (BME) supplemented with 4% bovine serum albumin (BSA). Individual petri dishes were examined at times which corresponded to 130, 175 and 185 h post-ovulation.

Influence of a collagen substratum on postblastocyst embryo development in a serumless medium*

Substratum	Hours post-ovulation	Hatching from the zona pellucida (%)	Attachment to the substratum (%)
Plastic	130	44/100 (44)[a]	0/44 (0)[a]
	175	69/100 (69)[b]	4/69 (6)[a]
	185	69/100 (69)[b]	0/69 (0)[a]
Collagen	130	14/52 (27)[a]	7/14 (50)[a]
	175	33/52 (64)[b]	27/33 (82)[b]
	185	33/52 (64)[b]	27/33 (82)[b]

*Embryos cultured in Eagle's basal medium (BME) supplemented with 4% bovine serum albumin. Each time was replicated 3–5 times. Percentages with different superscripts are significant at the $p < 0.05$ level using a statistical analysis which tests a hypothesis between population proportions.

The results are presented in tabular form. Embryonic development for each observed event was expressed as the rate of embryos that reached a particular stage of development to the total number of embryos that possessed the potential to reach that stage. The stages of development examined were a) hatching from the zona pellucida and b) attachment to the substratum.

As seen in the table, the collagen substratum delays hatching from the zona pellucida at 130 h post-ovulation (i.e., 27 vs 44%). By 175 h post-ovulation, the embryos with a collagen substratum have hatched at a rate comparable to those with a plastic substratum (i.e., 64 vs 69%). This rate of hatching is maintained at 185 h. When hatching from the zona pellucida does occur, the presence of a collagen substratum is essential if attachment is to occur in a serumless medium. As shown in the table, little if any attachment of hatched blastocysts occurred on the plastic substratum (i.e., 0–6%) while by 185 h post-ovulation, 82% of the hatched blastocysts had attached to the collagen substratum. The results of this study indicate that a collagen substratum has an influence on the hatching and attachment of the postblastocyst mouse embryo when cultured in a serumless medium. Previous work[9,18,19] has shown that if the medium is supplemented with fetal calf serum, the mouse blastocyst will hatch, attach, demonstrate trophoblast cell outgrowth the differentiate on a plastic substratum. One function attributed to the serum has been to coat the plastic surface thus enabling cells to attach and spread. Although a nutritional role for serum cannot be ruled out, Witkowski and Brighton[20] indicate that this physical role of serum may be as important. Our results indicate that BSA cannot substitute for serum in fulfilling this physical role; hence, attachment on plastic falls far below that seen on collagen. However, a collagen substratum allows attachment to occur when BSA is the only protein present in the medium. It is possible that the collagen rather than serving as a suitable surface for attachment may contribute something to the metabolism of the developing embryo. However, Jenkinson and Wilson[13] have shown that blastocysts cultured in the presence of collagen but without being in direct contact with it undergo very little development; again suggesting that the effect is physical rather than metabolic.

Initally, the presence of a collagen substratum in this culture system caused a reduction in the hatching rate of blastocysts as compared to the plastic substratum. The reduction in hatching in the presence of collagen may be the result of incomplete collagen equilibration with the culture medium. An alternative explanation may be the presence of a non-dializable toxic contaminant present within the collagen which after several medium changes may be diluted to the point where it does not influence embryo development.

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