versus controls; but it is not known whether this viral proliferation was induced by dexamethasone per se, or was the result of the presence of a greater number of differentiated cells, as suggested by an in vivo study[12]. It would be of interest to study the effects, on type C virus production, of other differentiation-inducing agents, especially those which increase intracellular cyclic AMP levels in murine neuroblastoma[18].

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Elevated glucose levels influence in vitro hatching, attachment, trophoblast outgrowth and differentiation of the mouse blastocyst

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Summary. A glucose concentration of 3.5 mg/ml is optimal for in vitro embryo attachment and trophoblastic cell outgrowth. Raising the concentration above 3.5 mg/ml does not improve embryo culture and can at certain concentrations be detrimental to embryo development.

Post-blastocyst development of the mouse embryo to the egg cylinder and/or beating heart stage can be accomplished in vitro[2-4]. Relatively little information is available concerning the metabolic requirements of these embryos during this stage of embryogenesis. Spindle and Pederson[5] have reported that increased amino acids are beneficial while exogenous nucleosides had no influence[6]. We have previously reported that glucose is a necessary factor since reduced glucose levels can delay hatching from the zona pellucida and attachment to the substratum[7]. The basic culture media for post-blastocyst development has been modified tissue culture media. Most media contain a glucose concentration of 1 mg/ml. Little information is available concerning the beneficial or detrimental effects of elevated glucose levels on post-blastocyst embryo development. The objective of this study was to ascertain the affects of elevated glucose levels on the in vitro hatching, attachment, trophoblastic cell outgrowth and differentiation of the mouse blastocyst.

Superovulation was induced in random bred Swiss mice by the method of Gates[8]. 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. 4 days postmating blastocysts were recovered by flushing the excised uterus with 0.5 ml of modified Brinster's medium[9]. 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 layered with collagen reconstituted from rat tails as described by Ehrmann and Gey[10] and modified by Hsu et al.[11]. Individual petri dishes were equilibrated with 5 ml of the culture media 60 min prior to the introduction of the mouse blastocysts.

The control blastocyst culture media consisted of Eagle's basal medium (BME) supplemented with 10% fetal calf serum (FCS). The fetal calf serum was previously analyzed for glucose content and determined to contain 150 mg/ml. Since BME contains 1.0 mg/ml of glucose, when supple-

Influence of elevated glucose levels on postblastocyst embryo development*

Glucose concentration (mg/ml)	Hatching from the zona pellucida (%)	Attachment to the collagen substratum (%)	Trophoblastic cell outgrowth (%)	Differentiation to the egg cylinder stage (%)
2.5 (control)	58/ 80 (73)[a]	49/ 58 (85)[a]	40/ 49 (82)[a]	2/ 40 (5)[a]
3.5	125/184 (68)[a]	119/125 (95)[b]	109/119 (92)[b]	6/109 (6)[a]
4.5	65/123 (53)[b]	45/ 65 (69)[c]	24/ 45 (53)[c]	0/ 24 (0)[b]
6.5	99/151 (66)[a]	93/ 99 (94)[b]	73/ 93 (76)[a,d]	4/ 73 (6)[a]
11.5	81/105 (77)[a]	76/ 81 (94)[b]	67/ 76 (88)[a,b]	5/ 67 (5)[a]

* Embryos cultured in Eagle's basal medium (BME) supplemented with 10% fetal calf serum. Each concentration of glucose 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.

mented with 10% FCS the final glucose concentration is 2.5 mg/ml. Aliquots of this medium were taken and enhanced with glucose to yield a final glucose concentration of 3.5, 4.5, 6.5 or 11.5 mg/ml.

Pooled blastocysts were randomly assigned to the culture media and left undisturbed until examined 48 h later. Daily observations were then made at 24 h intervals until a period of 5 days post-culture was reached. 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 possed the potential to reach that stage. The stages of development examined were a) hatching from the zona follucida, b) attachment to the collagen substratum, c) trophoblastic cell outgrowth and d) differentiation of the inner cell mass to the egg cylinder stage.

As seen in the table, increasing the glucose concentration above the control value of 2.5 mg/ml can influence in vitro mouse embryogenesis. At 3.5 mg/ml there is a significant increase in the number of blastocysts attaching to the substratum as well as the number exhibiting trophoblastic cell outgrowth. At 4.5 mg/ml there was a significant decrease in the number of embryos reaching all stages of development. Raising the glucose concentration to 6.5 or 11.5 mg/ml did not induce any significant changes with respect to the control concentration and/or the concentration of 3.5 mg/ml. The reason for the marked reduction in embryogenesis at 4.5 mg/ml is not clear but may be related to altered membrane permeability at this concentration. The improvement of attachment and trophoblastic cell outgrowth at 3.5, 6.5 and 11.5 mg/ml reflects a higher glucose need at these stages of development. Klebe[12] has previously reported that glucose is required for the in vitro attachment of cells to the substratum. It appears from this data that media which contain the routine concentration of 1.0 mg/ml of glucose do not allow optimum attachment or trophoblastic cell outgrowth. Raising the concentration above 3.5 mg/ml does not significantly increase the number of embryos reaching a particular developmental stage and may be harmful in some cases (i.e. 4.5 mg/ml). This research thus demonstrates that the routine concentration of glucose utilized for in vitro mouse embryogenesis at these developmental stages should be increased to a final concentration of 3.5 mg/ml. It also demonstrates that the glucose concentration of the fetal calf serum utilized for experiments of this type should be measured so that the experimentor has knowledge of the final glucose concentration in the experimental media.

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Electron microscopic observation of nucleosomes in ultrathin sections of heparin-treated nuclei

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Summary. Isolated rat liver nuclei display nucleosomes (γ-bodies) in common ultrathin sections after treatment of nuclei with heparin. The masking of nucleosomes in intact chromatin by some extranucleosomal component is suggested.

There is considerable evidence that chromatin is composed of nucleoprotein particles named γ-bodies[1] or nucleosomes[2]. The majority of investigations, including electron microscopic visualization, were performed on the sheared chromatin or isolated nucleosomes. The nucleosomes have not been distinguished, as a rule, in embedded material[3]. The only known exclusions are the nuclei with extensive chromatin decondensation, that display chromatin subunits in untreated ultrathin sections[4,5]. It therefore seems likely that pretreatment of nuclei with agents, causing loosening of chromatin, may be a useful method for visualization of nucleosomes. This study deals with such an attempt. For chromatin decondensation, we have used heparin - a natural polyanion, which is known to discharge histones from their complex with DNA[6].

Material and methods. Rat liver nuclei were isolated by the sucrose method[7]. The nuclear pellets were treated with 0.05% sodium heparinate diluted in isotonic saline (pH 7.0) at room temperature for 2-10 min. The nuclei were then fixed by adding glutaraldehyde to the incubation medium to the final concentration of 2.5%, for 1 h, postfixed with osmium tetroxyde (2%) for 2 h, block-stained with uranyl acetate during dehydration and embedded in Epon. Ultrathin sections were made with LKB ultratome, contrasted again with uranyl acetate and lead citrate according to

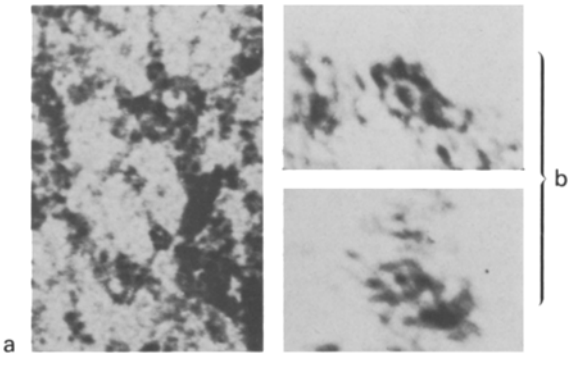

Fig. 1. Nucleosomes in rat liver nuclei after heparin treatment. *a* Many nucleosomes with connective threads. × 300,000. *b* Nucleosomes with the remnants of some extranucleosomal material. The central granule of nucleosome is seen. × 600,000.