Dietrich, 1976); however, background staining is high and variable. Alcian Blue with 0.05 M MgCl₂ provided effective labelling of all cartilage glycosaminoglycans, including trace amounts of hyaluronate. The hyaluronate did not form a complex with Alcian Blue either in the absence of MgCl₂ or at molarities greater than 0.1. In the described scheme, hyaluronate was resolved from chondroitin and keratan sulphates that migrated together (Fig. 1). Cartilage keratan sulphate (type II) was not a substrate of chondroitin ABC lyase (Yamagata et al., 1968), and could be recovered after subsequent electrophoresis and staining (Fig. 1). The chondroitin sulphates were determined from the difference before and after treatment with chondroitin ABC lyase, or alternatively by assay of the disaccharides (Elliott & Gardner, 1977, 1978). The calibration curves of glycosaminoglycan standards were in agreement with Whiteman's (1973) findings, although the assay procedures differed considerably.

The described procedure enables microgram quantities of cartilage glycosaminoglycans to be determined, by using microscopic sections, from anatomical defined zones of heterogeneous connective tissues, such as articular cartilage (Elliott & Gardner, 1979).


Lactate production and utilization by a differentiated culture of sheep thyroid cells

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Conventional primary cultures of thyroid cells are known to be capable of differentiation, with the formation of characteristic follicles (Fayet & Havegian, 1971). When regular changes of the supporting medium are made, cultures revert to an epithelial-like monolayer after approx. 5–8 days. O'Conor et al. (1979) demonstrated that, in the absence of medium changes, cell differentiation was retained until cell death occurred at about 15–20 days. By using 'conditioned medium' obtained from 10–14-day-old cultures, differentiated-cell life could be extended to at least 30 days (O'Conor et al., 1979).

Among the factors that could be responsible for cell death are the depletion of energy substrates or the accumulation of waste products; since a major source of energy in tissue-culture medium is glucose, it was decided to study glucose utilization and lactate accumulation over the culture life-span.

Methods of tissue culture have been described in detail elsewhere (O'Conor et al., 1979). Cells were maintained in Eagle's basal medium with 20% (w/v) lamb serum, 100μM/ml, units of thyrotropin and other additives. The system was buffered with a CO₂/air (1:19)/bicarbonate (Paul, 1975) system and maintained at near-physiological pH (range 7.25–7.45) until cell death was about to occur. Glucose (Schmidt, 1961) and lactate (Gutmann & Wahlefeld, 1974) were measured by using standard enzymic methods on HClO₄ extracts of frozen samples of medium.

Fig. 1 shows the utilization of glucose and the production of lactate by cultured cells over a typical experimental period in the presence of added thyrotropin. The glucose is broken down at the rate of 4–6 μmol/2 × 10⁵ cells per 24 h over a 5–9-day period. This results in the accumulation of what is almost a stoichiometrically equivalent amount of lactate. The lactate concentration reaches a peak when the glucose is exhausted.

At about this time the lactate begins to disappear from the medium at a similar rate. Cell death invariably coincides with exhaustion of the lactate supply. Qualitatively similar results have been obtained with several other media. In the absence of thyrotropin, glucose utilization and conversion into lactate were still observed, although the magnitude of the process was less. Dumont (1971), using dog thyroid tissues, has also reported that thyrotropin stimulates both glycolysis and lactate production.

Comparison of thyroid cultures with identically maintained CHO-K1 epithelial cells showed that the latter produce lactate, but not to the same extent as thyroid cells. They also tend to die when the glucose supply in the medium is exhausted (about 8 days).

It would appear that, although lactate production can occur in cell culture in general, the thyroid cells in this system are capable of living for 8–12 days after the disappearance from the medium of their major added energy substrate (glucose). It is possible that lactate is being used as an energy source, although the rate of use makes it difficult to suggest a mechanism. Lactate use has been reported for a few isolated cell types (Cribbs & Kline, 1971; Bailey et al., 1959; Molinary et al., 1968) and can occur at the same rate under aerobic and anaerobic conditions (Molinary et al., 1968).

No satisfactory mechanism has been elucidated, but Siegel & Bernlohr (1979) suggest that lactate has a regulatory role, whereas Molinary et al. (1968) showed that in tissue culture it is an important substrate for production of reduced nicotinamide nucleotides. This latter function may be particularly important in relation to the culture of thyroid cells, since NADH or NADPH is thought to be required for the peroxidase-mediated iodination of thyroglobulin, without which thyroid-hormone synthesis cannot take place (DeGroot & Stanbury, 1975).

The requirement of thyroid cells for an NADH source may explain why frequent changes of medium when using 'con-
Diotioned media rich in lactate promote differentiation of thyroid cells, whereas de-differentiation occurs with unconditioned medium containing no lactate.

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Differential uptake of a macromolecule by yolk sac and embryo of early rat conceptuses cultured in vitro

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The rat visceral yolk sac cultured in vitro has been used extensively in studies on the mechanism, substrate specificity and pharmacology of pinocytosis (Williams et al., 1975a,b, 1976; Roberts et al., 1976, 1977; Ibbotson et al., 1977; Pratten et al., 1978; Duncan & Lloyd, 1978; Duncan et al., 1979; Ibbotson & Williams, 1979; Livesey & Williams, 1979; Brown & Segal, 1977; Brown et al., 1979). In all these studies, yolk sacs were taken from pregnant rats at 17.5 days of gestation. Pinocytosis by the rodent visceral yolk sac has been postulated to have a nutritional function in the period between implantation and the development of a functional chorioallantoic placenta; it has been proposed that macromolecules captured pinocytically are digested within yolk-sac lysosomes and the products passed to the growing embryo (Williams et al., 1976).

We therefore decided to study pinocytosis by the rat visceral yolk sac at this early stage of development, which is, incidentally, the principal organogenetic phase and thus the most susceptible to teratogenic insult.

A rat egg cylinder is now available for culturing the 9.5-day rat egg cylinder, dissected free from surrounding tissue and denuded of Reichert’s membrane with the adherent parietal yolk sac, in rotating bottles containing heat-inactivated rat serum (New et al., 1973; Cockcroft, 1977). The egg cylinder is initially 1–2 mm long and, in cultures up to 48 h duration, embryonic development parallels development in vivo, so that embryos at harvesting display characteristic rotation and somite number 25–28 and have completed neural-tube closure.

Cultures of 48 h duration have been performed in which 125I-labelled polyvinylpyrrolidone was present, at a concentration of 2.8 µg/ml, in the culture serum for 1–24 h before harvesting. No deviations from normal development were seen. Embryos and yolk sacs were then separated, washed with Hanks (1948) medium and assayed for protein content (Lowry et al., 1951) and radioactivity. No radioactivity above the background value was found in the embryos even in those cultures where 125I-labelled polyvinylpyrrolidone was present for 24 h. In contrast, radioactivity was detectable in the yolk sacs in amounts that varied with the duration of exposure to the radiolabel. Measurement of protein content indicated that both yolk sac and embryo grow exponentially during the 48 h culture period, and the relationship between the time at which 125I-labelled polyvinylpyrrolidone was added to the culture and the amount of radioactivity found in the yolk sac conforms to a theoretical model that takes into account the increase in tissue size during the culture period. Further experiments using 125I-Hextran as substrate for pinocytosis have confirmed that uptake into the yolk sac does, but into the embryo does not, occur.

These experiments demonstrate that macromolecules, at least, cannot penetrate into the rat embryo at 9.5–11.5 days of gestation. They also confirm that the yolk sac is pinocytically active at this stage, as previously shown in vivo with horseradish peroxidase as substrate (Beck et al., 1967). 125I-Labelled polyvinylpyrrolidone is non-digestible by lysosomal enzymes, and further studies are required to demonstrate whether proteins captured by the yolk sac are digested and their component amino acids transferred to the embryo.

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