These results support the conclusion that acetaldehyde derived from metabolic conversion of ethanol acts as a non-specific inhibitor of liver monoamine oxidase.

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Production of Hyaluronate and Collagen by Fibroblast Clones in Culture

The synthesis of hyaluronate and of collagen are two specialized functions of connective tissue cells. Hyaluronate has been shown to be produced by populations of cultured fibroblasts by Grossfeld *et al.*[1], Morris[2], Castor *et al.*[3], and Davidson *et al.*[4], and collagen synthesis has been examined in cultured explants containing fibroblasts by Fitton-Jackson and Smith[5], Porter and Pappas[6] and Yardley *et al.*[7]. Both products have been shown to be synthesized in embryo heart explants by Gaines[8]. It has not been possible to decide, however, whether individual fibroblasts exist as distinct fixed cell types[10] specialized to form either polysaccharide or collagen[9], or if the same fibroblast type may make both products, perhaps under different environmental conditions[9].

The recent development of established fibroblast lines having infinite growth potential, but retaining their ability to synthesize collagen[11,12] and hyaluronate, has made it possible to examine these synthetic functions in cloned populations. One of these lines, 3*T*6, has a plating efficiency of about 20 per cent in the absence of feeder cells, grows with a doubling time of 13 h until saturation density is attained and then begins to secrete collagen at a constant rate[13], the protein being deposited between the cells as typical fibrils[12].

A confluent culture of this cell line was trypsinized, dispersed to single cells and Petri dishes were inoculated with very dilute cell suspensions, so that not more than two or three widely separated colonies grew out in each plate. These colonies were isolated in cloning cylinders by the method of Puck, Marcus and Cieciura[14], transferred, and grown out to mass cultures. No larger than average colonies were selected to avoid the possibility of a colony having actually arisen from a clump containing more than one cell. Five clones were isolated and grown out to mass culture. Cultures derived from each of the clones were allowed to remain at saturation density without transfer for periods up to 11 days, the medium being changed three times weekly.

Samples of medium and of the cell layer of saturation density cultures were assayed for hexuronic acid by a modification of the method of Sundblad[15]. Medium or cells homogenized in saline by grinding with glass beads were dialysed against buffer *p*H 4·8 (0·1 M sodium chloride and 0·05 M acetate), and digested with testicular hyaluronidase for 24 h at 37°. Protein was then precipitated with trichloroacetic acid at a final concentration of 5 per cent, and hexuronic acid in the supernatant determined by the carbazole reaction[16].

Hexuronic acid containing polysaccharide was found in the cell layer and in the medium of cultures of each of the clones tested. Streptococcal hyaluronidase was as effective as testicular hyaluronidase in degrading the polysaccharide in the medium; the polysaccharide may, therefore, be assumed to consist almost exclusively of hyaluronate. Assuming a hexuronic content of 40 per cent, it was calculated that the clones secreted into the medium 100–200 μg of hyaluronate per 10^7 cells per day. This rate of production is in the range reported by Castor *et al.*[3] for human fibroblasts derived from several organs, and by Morris[2] for a rat fibroblast line.

Cell layers of duplicate cultures were washed, scraped from the Petri plates into 6 N hydrochloric acid and hydrolysed at 120° for 10 h. Hydroxyproline determinations were performed by the method of Prockop and Udenfriend[17]. Each clone was found to accumulate collagen in the cell layer of saturation density cultures at the same time that hyaluronic acid was secreted into the medium. Assuming a constant rate of formation after saturation density was attained[13] and a hydroxyproline content of 13 per cent, the rate of collagen formation was estimated to range from 5–16 μg per 10^7 cells per day. On the average, then, the various clones of 3*T*6 appear to synthesize the polysaccharide at roughly 15 times the rate at which they synthesize collagen.

It may be concluded that hyaluronate formation and collagen synthesis both occur in homogeneous cultures of 3*T*6 descended from single cells and are, therefore, functions of the same cell type. In all probability both functions are carried out simultaneously in each cell of saturation density cultures.

Another fibroblast line, 3*T*3, in the course of its establishment appears to have lost its ability to synthesize collagen[11] but continues to synthesize hyaluronate at the same rate as 3*T*6. No line has been found which is able to synthesize collagen but not hyaluronate, though a systematic search has not been made.

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A Possible Role of Phosphate in Regulating Phosphatase-level in the Rat Kidney

The part played by phosphate in repressing alkaline phosphatase formation has been described in *E. coli*[1–3]; the first results of research on the possibility of an analogous regulation in rat kidney are presented here.

The effect of administering to rats a diet lacking in phosphate on the levels of alkaline phosphatase, inorganic phosphate and a possible substrate of the enzyme, glycerol-1-phosphate, in the kidney, was investigated.