Tyrosine Transaminase Induction by Dexamethasone in a New Rat Liver Cell Line

Abstract. *A cell line derived from normal, adult rat liver has been established; the cells are similar to hepatocytes, as shown by electron microscopy. The addition of dexamethasone to the culture medium induced a three- to sixfold increase in the specific activity of tyrosine α-ketoglutarate transaminase; this increase was inhibited by the simultaneous addition of cycloheximide or actinomycin D. The latter, when added to cells given prior treatment with dexamethasone, further enhanced the transaminase activity. Contact-inhibited cells showed a lower response to dexamethasone than exponentially growing cells.*

A technique for culturing liver cells from newborn rats has been described (*1, 2*). Because the enzymic pattern of rat liver changes after birth (*3*), we have developed a technique for culturing adult rat liver cells since these cells have a more stable enzyme pattern. We describe here some of the morphological characteristics of the new adult rat liver cell line and some experiments on the induction of the hepatic enzyme tyrosine α-ketoglutarate transaminase (TKT) (E.C.2.6.1.5.) by dexamethasone sodium phosphate (*4*) added to the culture medium. The induction of this enzyme by adrenocortical hormones has been demonstrated in hepatoma cells in culture (*5, 6*); in sodium tetraphenylborate-dispersed rat liver cells (*7*); and in fetal rat liver in organ culture (*8*). Similar results have been reported in 30-day-old cultured rat liver cells obtained from newborn rats (*2*); however, no such report has been made, to our knowledge, involving a cell line obtained from normal adult rat liver.

The cell line (RLC) described here was obtained from the liver of a male Wistar rat (175 g). The liver was dissociated on 1 May 1969 according to a previously described procedure (*2*), except that a lower concentration of sodium tetraphenylborate was used [$5 \times 10^{-5} M$ in potassium-free Eagle's minimum essential medium (*9*)]. The cells were first cultured in suspension at a concentration of 2×10^4 cells per milliliter for 5 months and then transferred to monolayer culture in plastic containers (Falcon Plastic). The culture medium employed was Ham's F-12 (*10*) supplemented with 10 percent calf serum. The cultures were maintained at 37°C in a humidified atmosphere of CO_2 and air (5 : 95). Cells in monolayer culture were given a total change of medium every 2 days, while cells in suspension culture were centrifuged at 50*g* for 10 minutes and then given a 50 percent change of medium every 2 days. Cellular growth rate was logarithmic with a doubling time of 2 days in monolayer culture and 4 days in suspension culture. The RLC cells possess the histological characteristics of epithelial cells. At one point the cells were frozen in a mixture of glycerol and Ham's medium (10 : 90) (*11*) and were maintained in liquid nitrogen; after the cells were thawed they exhibited the same morphology and growth pattern as before. Several clonal cell lines have been obtained from the original strain, and these cloned cells showed a morphology and growth pattern similar to those of the parent cell population.

The chromosome number varied from 57 to 61, as judged by standard techniques (*12*). The electron microscopy studies were performed on cells grown in monolayer after the following preparative procedure. The medium was removed, and the cells were fixed with phosphate-buffered glutaraldehyde (1 percent) for 1 hour at 4°C. The cells were washed with the same buffer and then fixed again in the phosphate-buffered glutaralde-

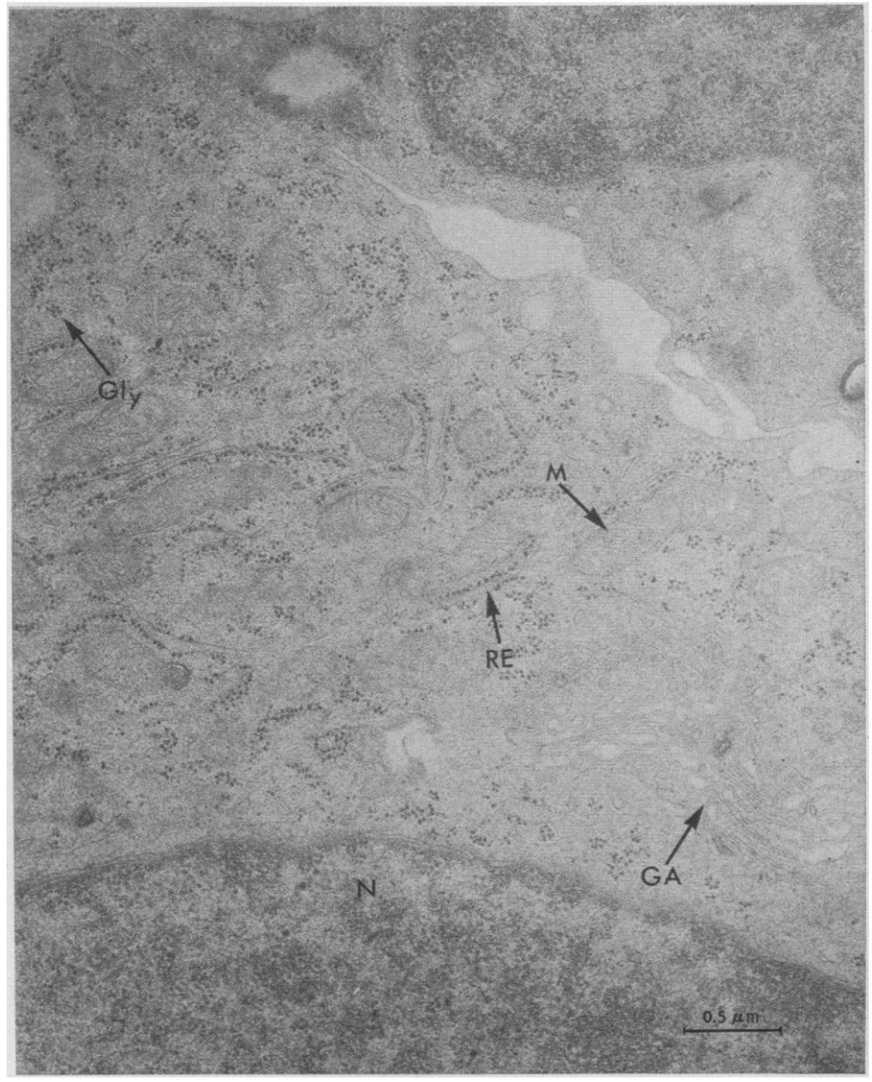

Fig. 1. Electron micrograph (Siemens Elmiscop 1A) of RLC cells. For technique used, see text. Abbreviations are: *N*, nucleus; *GA*, Golgi apparatus; *RE*, rough endoplasmic reticulum; *M*, mitochondria; and *Gly*, glycogen.

hyde for 1 hour at 4°C. After a second washing with buffer, the cells were dehydrated in ethanol and propylene oxide, embedded in Epon, and sectioned on a Sorvall MT-2 microtome with a diamond knife. The sections were stained first with a saturated aqueous solution of uranyl acetate and then with Reynolds' lead citrate preparation (*13*). The micrograph shows (Fig. 1) mitochondria with scanty cristae, rough endoplasmic reticulum, Golgi apparatus, and glycogen deposits irregularly scattered throughout the cytoplasm. Desmosomes have been observed at points of contact between cells.

The basal activity of TKT varied from 5 to 7 units (expressed as the number of nanomoles of the complex or *p*-hydroxyphenylpyruvateenol and borate formed per minute per milligram of protein); the addition of $10^{-5}M$ dexamethasone induced a three- to sixfold increase in enzyme specific activity. The activity of TKT in cells cultured without the hormone did not change (Fig. 2). When dexamethasone was added to the medium, the specific activity of the enzyme increased rapidly, reaching a plateau after 18 hours and remaining constant unless the dexamethasone was removed. After the dexamethasone was removed, TKT specific activity decreased to basal amounts within 25 hours. The simultaneous addition of actinomycin D or cycloheximide with the hormone inhibited its effect. When actinomycin D was added after prior treatment of the cultured cells with the hormone, the enzyme activity was enhanced even further in the presence or absence of dexamethasone. A similar effect of actinomycin D on the TKT of hepatoma cells in culture has been reported (*5, 6*). The addition of cycloheximide in conjunction with the removal of dexamethasone slowed down the rate of decrease of the enzyme activity. The concentration of cycloheximide used inhibited 90.6 percent of the incorporation of [^{14}C]-leucine into proteins. A similar effect of protein synthesis inhibitors in decreasing the rate of enzyme degradation has been shown in whole animals (*14*). In our experiments cell density was an important factor influencing the magnitude of the cellular response to the hormone. Contact-inhibited cultures showed only half the dexamethasone-induced increase in TKT activity that was seen with exponentially growing cultures (Fig. 3). During the first week of stationary phase the cells are perfectly viable; they do not peel off, as may happen later, and they can be easily subcultured with a plating efficiency of over 70 percent. Because the incubation of cells in either growth phase with medium removed from cultures in the other growth phase produced no effect on the relative degree of dexamethasone stimulation of TKT activity between contact-inhibited and exponentially growing cultures, we eliminated the following possible explanations for this phenomenon: (i) the secretion of an inhibitor of TKT synthesis by contact-inhibited cells; (ii) the secretion of an activator of TKT synthesis in actively dividing cells; and (iii) the rapid depletion or destruction of a putative factor in the medium essential for the action of dexamethasone. The effect of the addition or removal of dexamethasone or the inhibitors of macromolecule synthesis, or both, on

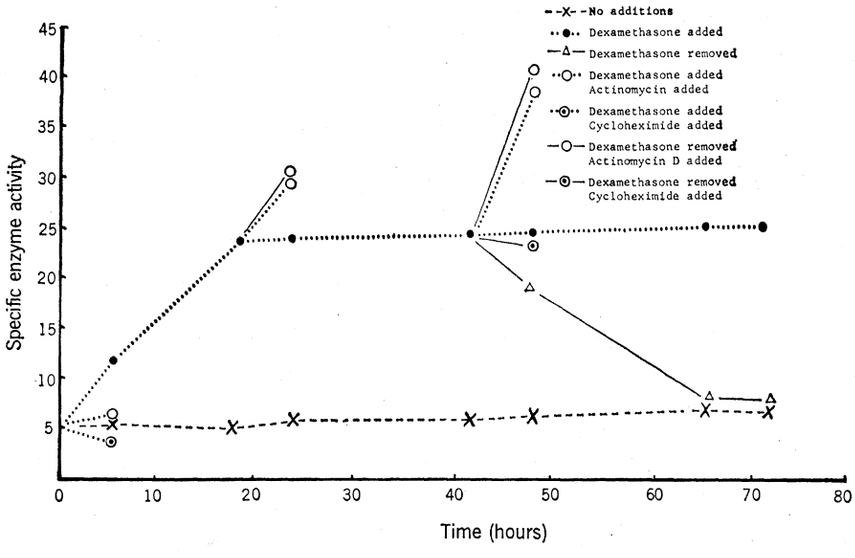

Fig. 2. The effect of $10^{-5}M$ dexamethasone sodium phosphate on tyrosine α-ketoglutarate transaminase of RLC cells cultured in monolayer in plastic tissue-culture flasks (75 cm^2). To start the experiment, the culture medium was removed and 20 ml of fresh medium with or without additions was added to each flask. When dexamethasone was removed, the medium was poured off; the cells were washed with 10 ml of fresh medium, and 20 ml of new medium with or without additions was added. The concentrations of actinomycin D and cycloheximide used were 0.25 and 28 μg/ml, respectively. The incubation was carried out at 37°C under the same conditions as the regular cultures. Flasks were removed at various times; the cells were washed twice with 10 ml of Hanks saline solution (*19*) and were removed from the flasks by the use of a rubber policeman. The resulting cell suspension was centrifuged at 300*g* and washed once with 0.15*M* sodium phosphate buffer, *p*H 7.8. The cells were then suspended in 1.5 ml of buffer and homogenized by means of a sonicator (two bursts of 7 amp for 10 seconds each). The homogenate was centrifuged at 16,000*g* for 15 minutes. Portions of the supernatant were assayed for TKT by the Tomkins *et al.* (*5*) modification of the method of Lin and Knox. The light absorption at 310 nm was followed with a Gilford recording spectrophotometer. The results are expressed as specific activity: the number of nanomoles of the complex *p*-hydroxyphenylpyruvateenol and borate formed per minute per milligram of protein with the molar absorbancy of the complex taken to be 10,700. Protein was determined by the procedure of Lowry *et al.* (*20*).

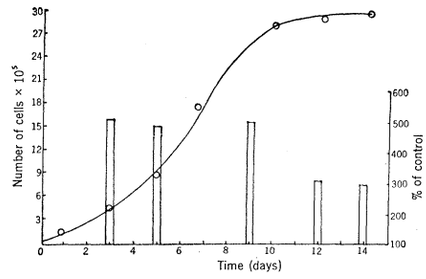

Fig. 3. Effect of dexamethasone on tyrosine α-ketoglutarate transaminase of RLC cells measured at different cell densities. The experiment was carried out as described in Fig. 2; one flask was used to count the number of cells after trypsinization with a hemacytometer. Two other series of flasks with and without dexamethasone were used to determine the enzyme activity. The empty circles depict the growth, measured by counting the cells from parallel bottles in a hemacytometer; the bars indicate the percentage of increase of the enzyme specific activity over the control after 24 hours of incubation with the hormone.

basal enzyme activities and on the kinetics of the resulting variations in TKT activity was essentially the same for cells in both growth phases, the only observable difference being the decrease in the magnitude of the stimulation by dexamethasone. The effects on the basal enzyme activities, on the kinetics of the increase and decrease after addition or removal of dexamethasone, and on the inhibitors of RNA or protein synthesis were the same for cells in both growth phases, the only noticeable difference being the decrease in the magnitude of the increase. Nebert and Gelboin (*15*) described a similar phenomenon during the induction of aryl hydroxylase in cells in culture, and attributed that effect to a decreased rate synthesis of DNA, RNA, and protein in contact-inhibited cultures, as had been previously described (*16*). Although these latter findings may very well explain our results, the observed decrease in dexamethasone sensitivity may also be the result of an increase in the proportion of cells in early G_1 in a random cell population; the activity of TKT cannot be enhanced by dexamethasone in synchronized hepatoma cells in culture during the part of the cell cycle between the beginning of G_2 and the early part of G_1 (*17*).

It is important to maintain careful control of cell density in any study involving hormonal effects in cultured cells; recent experiments have also demonstrated that, whereas the enzyme pyruvate kinase is insensitive to insulin in contact-inhibited RLC cells, it is readily inducible in these cells during their logarithmic growth phase (*18*).

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Scanning Electron Microscopy of the Organ of Corti

Abstract. *With the scanning electron microscope we have examined normal cochlear sensory epithelium of the guinea pig and cat and that damaged by noise. The studies demonstrate how the regular surface architecture of the organ of Corti is altered after exposure to noise. The changes include loss of sensory hairs, formation of giant hairs, and complete degeneration of circumscribed areas of the organ of Corti. Our method greatly reduces the artifacts.*

The application of scanning electron microscopy to the study of biological materials, including, for example, pollen, protozoa, enamel, and respiratory epithelia, has been reviewed by Hayes *et al.* (*1*), Pease and Hayes (*2*), Barber and Boyde (*3*), and Small and Marzalek (*4*). Scanning electron microscopy has very recently been applied to the inner ear, first by Barber and Boyde (*3*), and since by Lim and Lane (*5*), Engström *et al.* (*6*), and Marovitz *et al.* (*7*).

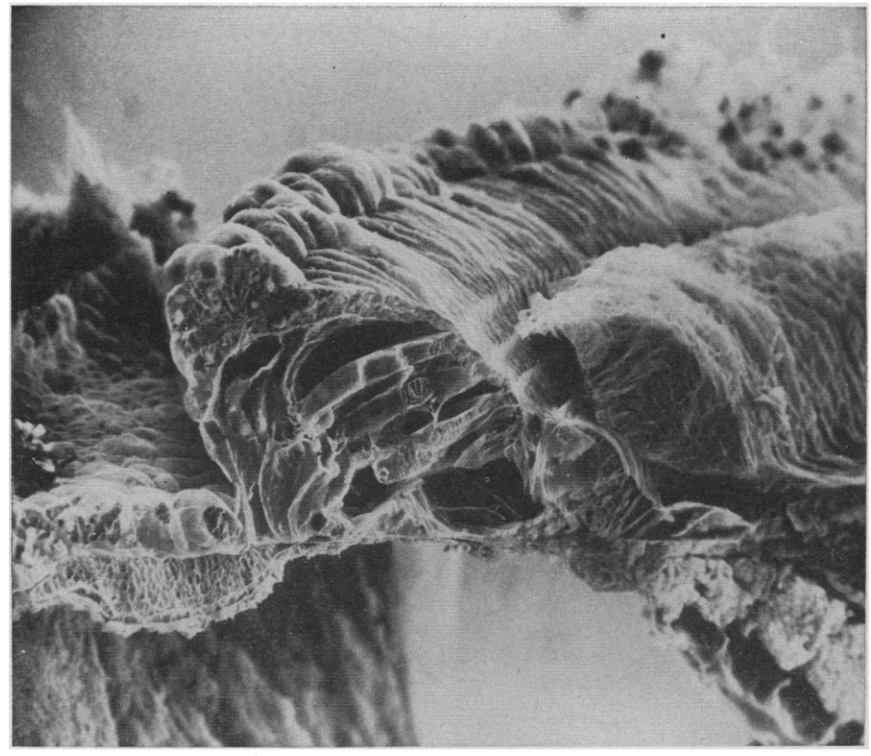

Fig. 1. Fracture through the organ of Corti of the guinea pig, showing the overall picture of the acoustic papilla. This specimen, prepared by fracturing the freeze-dried specimen, shows the possibility of studying the interior structures of the sensory epithelium (× 370).