ESTABLISHMENT OF A CLONAL STRAIN OF HEPATOMA CELLS WHICH SECRETE ALBUMIN

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ABSTRACT

A clonal strain of epithelial cells (designated MH_1C_1) has been established from the transplantable Morris hepatoma No. 7795. The cells have maintained distinctive morphology throughout more than 20 subcultures (split 1:5) at 2- to 4-week intervals in supplemented Ham's F 10 medium. They contain many highly refractile, round, cytoplasmic bodies which stain bright red with Oil Red O. The population doubling time was 2 wk when the clonal strain was first established. It has gradually decreased to 1 wk. The cells synthesize rat serum albumin and secrete it into the culture medium as determined immunologically by microcomplement fixation and double diffusion. Albumin secretion (3–6 μg albumin/mg cell nitrogen/24 hr) occurs throughout the logarithmic phase of cell proliferation and has not diminished during serial propagation since the strain was initiated 15 months ago.

INTRODUCTION

Animal cells propagated serially in culture rarely maintain the differentiated functions which are characteristic of the tissue from which the cells were derived (1). This circumstance can be ascribed in part to overgrowth of parenchymal cells by fibroblasts or other connective tissue cell types (2–4). Selective overgrowth by cells of connective tissue origin may be eliminated by single-cell plating and cloning procedures. With these techniques, several strains of "functional" mammalian cells have been established in culture (3, 5, 6). These cells continue to perform organ-specific function in culture even after serial propagation for many months.

This report[1] describes in detail the establishment and characterization of a clonal strain of rat hepatoma cells which perform a liver-specific function in culture. These cells synthesize rat serum albumin and secrete the protein into the culture medium. The cells have been grown in culture for 15 months and have shown no evidence of loss of specific function during this period.

MATERIALS AND METHODS

Origin of the Hepatoma

The tumor used to establish primary cultures was the Morris hepatoma No. 7795 (7). A tumor-bearing host animal was kindly donated by Dr. Peter Ofner, Lemuel Shattuck Hospital, Jamaica Plain, Massachusetts, and by Dr. Harold P. Morris, National Cancer Institute, Bethesda, Maryland. This hepatoma is transplantable in rats of the Buffalo strain.

[1] A preliminary report of these findings has been made (24).

Method of Culture

Primary cultures were made by direct plating of cells from the original tumor. The tumor was excised from the thigh, the capsule was opened, and a piece of tumor was placed in a sterile Petri dish. The tissue was minced finely with scissors. Viokase solution (0.2% in serum-free culture medium) was added, and the cell mince was dispersed further by repeated aspirations through a pipet. The Viokase solution was removed after centrifugation at low speed. The cell pellet was resuspended in culture medium, and the tube was allowed to stand for 5 min, during which time the larger tissue pieces settled out. Aliquots of the supernatant fluid containing single cells and aggregates of small numbers of cells were plated on Petri dishes (more than 10^6 cells/dish) and were incubated as described below.

Cultures were grown in plastic Petri dishes (60 × 15 mm) or in bottles (250 ml). They were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Synthetic culture medium, Ham's F 10 (8), was supplemented with 15% horse serum and 2.5% fetal calf serum. The medium was changed twice weekly on the Petri dishes, once weekly in the bottles. Cells were subcultured after incubation with 0.1% Viokase solution in phosphate-buffered saline for 5–10 min at 37°C (3). The Viokase solution was aspirated after low-speed centrifugation, and the cells were resuspended in culture medium. The suspension was diluted 1:5 to 1:20, and the cells were plated in individual Petri dishes or in bottles and incubated as described above.

Cloning Procedure

Two morphologically distinct cell types proliferated in primary cultures: large, flat, irregularly-shaped cells and small, round, epithelial-like cells. The latter cell type was selected for cloning by repeated mechanical isolation of homogeneous colonies derived from single cells, according to the methods of Puck et al. (9). The final clonal strain was designated MH_1C_1 for Morris hepatoma, clone one. This strain has been grown in continuous serial tissue culture for 15 months in the same medium and under the same conditions as described for primary cultures. Absolute plating efficiency for the clonal strain ranged from 1.5 to 2.5% when 0.5–5.0 × 10^3 cells were inoculated in a 60 mm plastic Petri dish.

Measurements of Growth and Function

Experiments designed to assess growth and function of the hepatoma cells were carried out on a group of identical plates established simultaneously from a homogeneous suspension of cells. At 1 wk intervals three plates from the group were chosen randomly. Culture medium from two plates was collected separately for immunoassay of rat serum albumin content (see below). Medium for immunoassay was stored at −20°C, and all assays were performed within 2 wk of collection of the medium; no loss of serum albumin was detected during this storage period. The plates from which the medium was saved were washed twice with 0.15 M NaCl and were also frozen at −20°C for the later determination of cell nitrogen. Cells from such replicate plates were scraped separately from each dish with the aid of two 1.5 ml aliquots of a 0.1 N NaOH solution. The cell suspensions were homogenized in an all-glass tissue grinder. Protein nitrogen in the cell homogenates was determined for each dish by the method of Lowry et al. (10). Albumin concentrations in medium and cell nitrogen determinations represent, therefore, mean values from duplicate plates.

Cells from the third Petri dish were detached with two 2 ml aliquots of Viokase solution and were collected in a final wash with 2 ml of 0.15 M NaCl solution. The suspended cells were counted in a hemocytometer after staining with crystal violet.

Organ-specific function of the hepatoma cells in culture was studied by examining both the cells and the culture medium for serum albumin, a product characteristic of normal liver. Rat serum albumin was measured immunologically by quantitative micro-complement fixation (11), by using rabbit antiserum to whole rat serum.

Rat serum albumin was obtained commercially (Pentex, Inc., Kankakee, Ill.) and purified further by repeated ammonium sulfate precipitations. There was no evidence of heterogeneity by analytical disc gel electrophoresis (12) or by double diffusion (13) with 13-fold concentrated antiserum to whole rat serum. The purified rat serum albumin served as the standard in the immunoassay. Samples of medium were prepared for assay by dilution (usually 1:3 or 1:5) in the complement (C′)-fixation buffer (0.15 M NaCl-0.01 M Tris, pH 7.4, containing gelatin, 1 mg/ml, and 1.5 × 10^{-4} M Ca^{+2} and 5 × 10^{-4} M Mg^{+2}). Heating the diluted samples at 60°C for 20 min was found to be effective in eliminating anticomplementary activity without affecting rat serum albumin (initially present or added) in the medium. Hepatoma cells suspended in 0.15 M NaCl were lysed by sonic oscillation for the assay of intracellular albumin.

Serum albumin synthesized by the clonal hepatoma cells was also characterized by the double diffusion technique of Ouchterlony (13), by using both undiluted and concentrated antiserum. Antiserum was concentrated by lyophilization followed by reconstitution in water to $\frac{1}{13}$ the original volume.

The results of growth and function experiments were expressed as follows. Three curves were plotted. One curve related cell nitrogen, a second curve

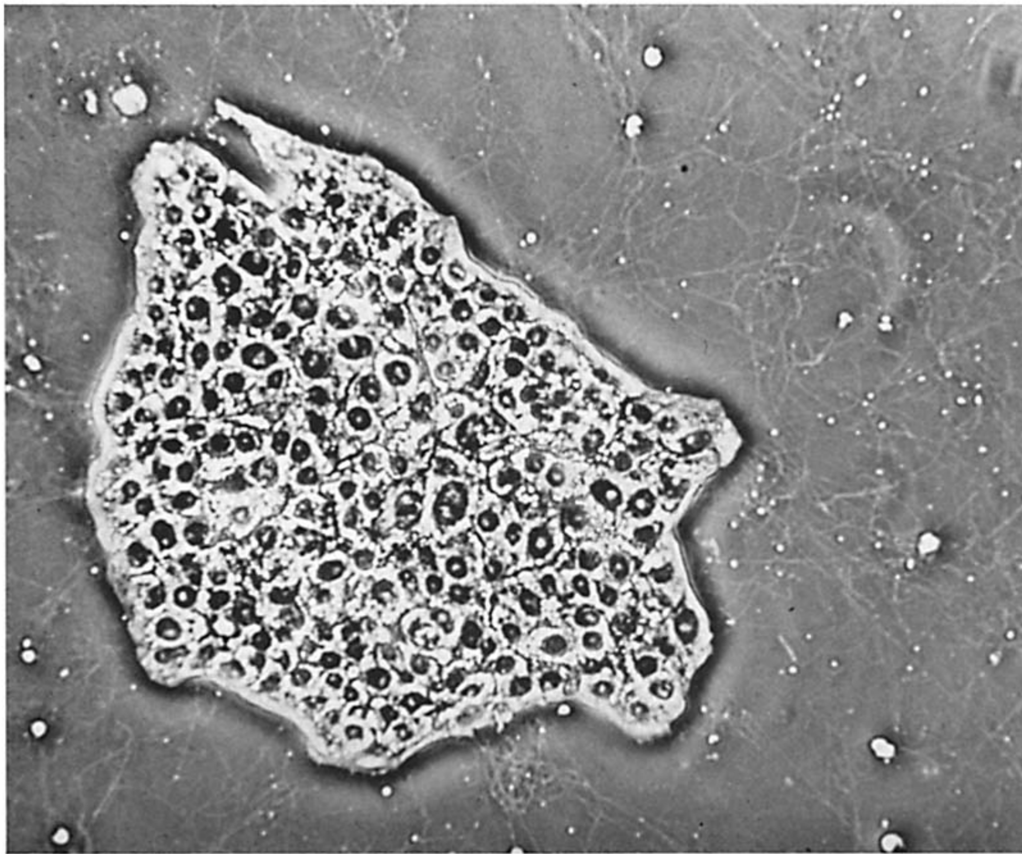

FIGURE 1 Photomicrograph of cells of the MH_1C_1 strain. This picture gives the appearance of cells 11 months after establishing the clone and after approximately 15 subcultures. Unstained, original magnification × 70, phase contrast.

related cell number, and a third curve related rat serum albumin in the medium to time in culture.

RESULTS

Appearance of a Conal Strain, MH_1C_1, of Rat Hepatoma Cells

Fig. 1 shows a colony of MH_1C_1 cells as it appears 3 wk after subculture. The border of a young colony is characteristically smooth, and the cells grow in a compact mass. Individual cells are small and rounded or polygonal in shape. Each cell has a prominent nucleus with one, two, and occasionally more nucleoli. The relatively scant cytoplasm contains numerous highly refractile bodies (Fig. 2). These bodies are particularly prominent in cells during the phase of logarithmic growth. They decrease in number as the culture ages. Many of these cytoplasmic bodies selectively take up the lipophilic stain, Oil Red O, presumptive evidence that they contain lipid material (Fig. 3).

Cells of the MH_1C_1 strain do not form a complete monolayer even after prolonged growth (60–80 days) without subculture. In cultures over 60 days old, the number of cells adhering to the surface of the Petri dish remains at approximately $1.2–1.5 \times 10^7$ for an additional 20–30 days. Since cells are continually shed into the medium in these older cultures, the area of the plate occupied by cells remains relatively constant and is generally less than 80% of the total surface area.

Determination of Organ-Specific Function in Clonal Hepatoma Cell Cultures

Studies by other investigators have shown that the particular tumor (Morris hepatoma No. 7795)

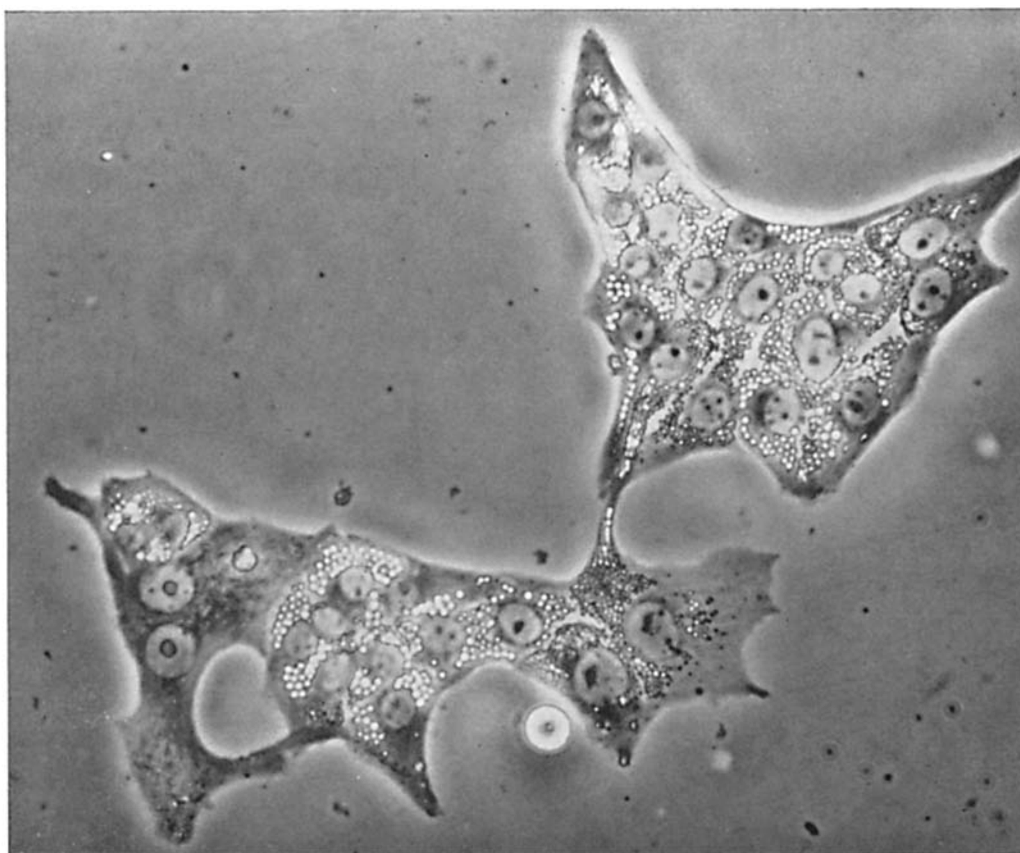

FIGURE 2 Photomicrograph of a small colony of cells of the MH_1C_1 strain. This picture gives the appearance of the cells 2 wk after subculture. Note the large number of refractile, round bodies present in the cytoplasm of the cells. These bodies are not of constant size and vary in number from cell to cell. Unstained, original magnification × 250, phase contrast.

from which the MH_1C_1 cell strain was derived possesses certain enzymes characteristic of normal liver (14–16). To investigate the possibility that the MH_1C_1 cells might perform liver-specific functions in vitro, both the cells and the medium were assayed immunologically for the presence of rat serum albumin by C′ fixation. To show that under the conditions used to perform the assays rat serum albumin was the only antigen measured, C′-fixation curves were obtained with purified rat serum albumin, with whole, diluted rat serum, and with 3-day-old medium from an MH_1C_1 culture (Fig. 4). The C′-fixation curves obtained with purified rat serum albumin and with rat serum were essentially superimposable; equal amounts of C′ were fixed at the zones of antigen-antibody equivalence, indicating qualitative similarity between the antigen being measured in whole rat serum and authentic rat serum albumin. If the two antigens were different but related molecules, or if two different immune systems were being detected, the amounts of C′ fixed at equivalence would not be the same for the two curves (17). The dilution of rat serum giving peak C′ fixation was 1:730,000, equivalent to 65 mμg albumin. This concentration of antigen was, within the limits of error for the assay method, the same as that giving peak fixation with purified rat albumin. As shown in Fig. 4, medium from cultures of MH_1C_1 cells fixed about 15% less C′ at the zone of antigen-antibody equivalence than did either rat albumin or rat serum. Because MH_1C_1 medium fixed less C′ with antibody than did rat albumin or rat serum, it was not possible to know with certainty from this experiment whether the antigen measured in medium from MH_1C_1 cells

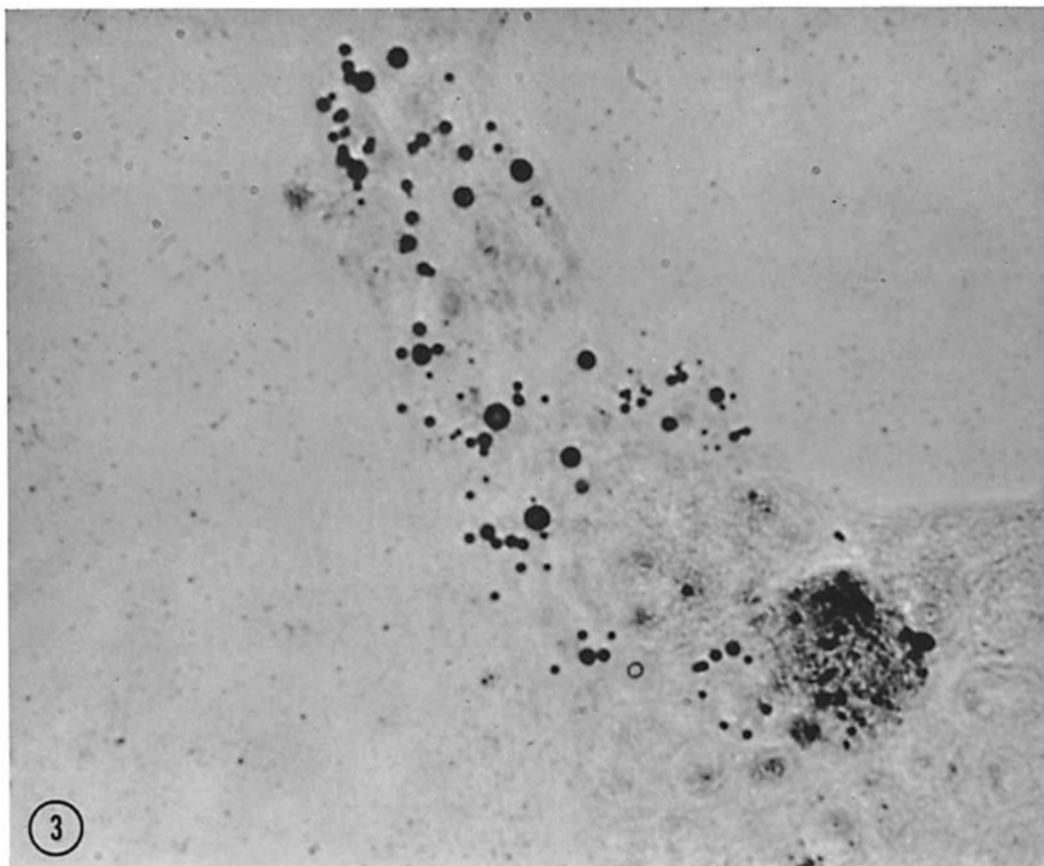

FIGURE 3 Photomicrograph of cells of the MH_1C_1 strain after staining with Oil Red O. The culture was prepared for staining by washing the cells two times with 0.15 M NaCl. A solution of Oil Red O (0.5%) in isopropanol was added to the culture dish and it was then allowed to stand for 5 min. The stain was removed and the cells were washed with 0.15 M NaCl. The stained culture was examined microscopically with both bright-field and phase contrast optics. The only intracellular cytoplasmic structures which took up the stain were the previously visible refractile bodies. They stained bright red but appear black in this photomicrograph. The cells are not in focus because the phase objective and condenser were purposely mismatched in order to emphasize the contrast of the stained structures. Occasionally, several small bodies appeared to coalesce during the staining procedure to give a single, large, stained droplet. In control experiments several other cell lines which did not contain intracellular cytoplasmic refractile bodies were treated with Oil Red O. No staining was observed in these control cells. Original magnification × 500, phase contrast.

was truly rat serum albumin, a modified albumin molecule, or some other substance. Therefore, additional experiments were performed to determine the reason for the depression in the C′-fixation curve.

Fig. 5 shows C′-fixation curves obtained with rat serum alone and rat serum added to fresh, uninoculated medium. In the presence of medium the amount of C′ fixed at equivalence with rat serum was decreased 15%. The same result was obtained when purified rat albumin was assayed in the presence of fresh medium. These experiments were repeated four times (two times with duplicate reaction mixtures). In the presence of culture medium the C′-fixation peak was depressed 13–16%, but there was no lateral shift in the zone of equivalence along the abscissa, indicating no quantitative change in the amount of

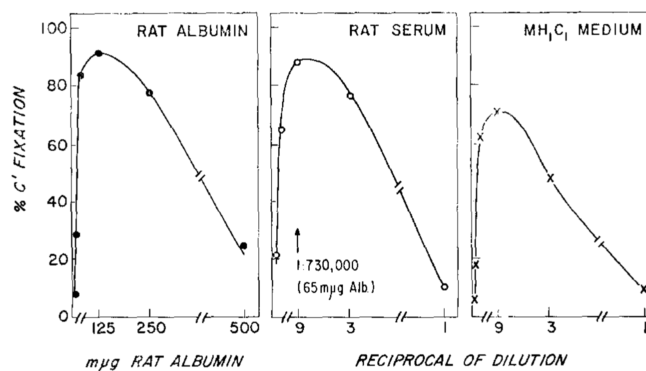

FIGURE 4 C′-fixation curves obtained with purified rat serum albumin (left), diluted whole rat serum (middle), and medium from MH_1C_1 cells and rabbit antiserum to whole rat serum (diluted 1:1,500). The original (1:1) dilutions of rat serum and MH_1C_1 medium were 1:81,000 and 1:4, respectively. If some substance other than albumin were being measured in rat serum, the amount of C′ fixed at equivalence would have been different from that obtained with purified albumin; and in addition, a lesser dilution of rat serum would have been required to reach equivalence with a serum component less abundant than albumin.

antigen being measured. Uninoculated medium alone did not fix C′ directly with antiserum.

Fig. 6 shows that, although fresh, whole medium or its protein components (horse serum and fetal calf serum) did not fix C′ directly with antiserum, they did react with antirat serum as evidenced by inhibition of the rat serum-antirat serum immune system. This inhibition was specific as shown by the lack of inhibition of three other immune systems (rat growth hormone-antirat growth hormone, human growth hormone-antihuman growth hormone, and ovine prolactin-antiprolactin) by fresh medium, horse serum, or fetal calf serum. Similar results were obtained when purified rat albumin was used in place of diluted rat serum as the antigen in the C′-fixation inhibition experiments. These data reveal a weak immunological cross-reaction between an antigen, presumably albumin, in horse serum and fetal calf serum and antiserum to rat serum. The major inhibitor in whole fresh medium was probably horse albumin, since the concentration of horse serum in medium was 15% as compared to 2.5% for fetal calf serum.

These results show that the decrease in the amount of C′ fixed at equivalence with rat serum albumin in the presence of whole culture medium is due to weak cross-reactions of antirat albumin with horse and fetal calf albumins. A small fraction of the antirat albumin binds (Fig. 6), but does not fix C′ directly, with horse and fetal calf albumins and is, therefore, not available for reac-

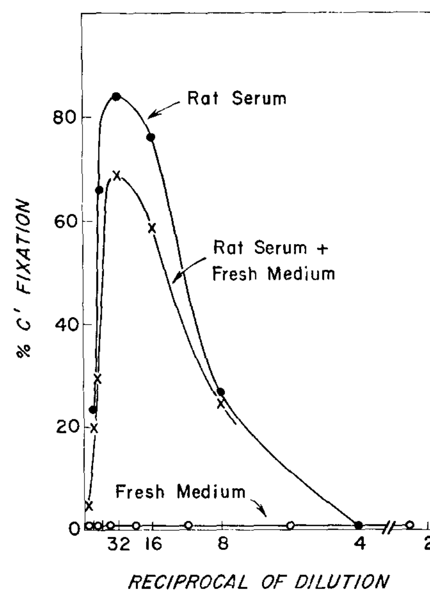

FIGURE 5 C′-fixation curves obtained with rabbit antiserum, diluted 1:1,500, and rat serum alone (●) or rat serum added to fresh culture medium (×) immediately before serial dilution and addition to the reaction tubes. Original dilutions (1:1) of rat serum and fresh medium were 1:40,000 and 1:3, respectively. The original dilution (1:1) of rat serum in fresh medium was 1:40,000 in 1:3 medium. Fresh medium did not fix C′ directly with antiserum. Identical results were obtained when purified rat serum albumin was used in place of diluted rat serum.

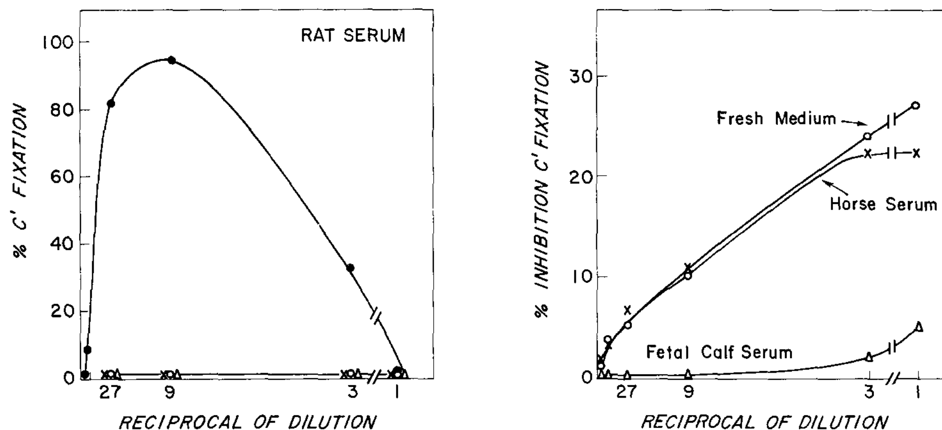

FIGURE 6 On the left is shown the C′-fixation curve obtained with rat serum and rabbit antiserum (diluted 1:1,500). At this dilution of antiserum no direct C′-fixation was seen with whole fresh medium (○), horse serum (×), or fetal calf serum (△). On the right are shown C′-fixation inhibition curves. The C′ fixation being inhibited was that obtained with rat serum, diluted 1:800,000, and antiserum, diluted 1:1,500. The original dilution (1:1) of fresh whole medium was 1:3. The original dilutions (1:1) of horse serum and fetal calf serum were equivalent to concentrations of those materials in a 1:3 dilution of fresh whole medium.

tion with the homologous antigen, rat albumin. This small decrease in available antibody is, in turn, responsible for the observed decrease in the amount of C′ fixed at antigen-antibody equivalence (17) when the antigen is rat albumin added artificially to culture medium (Fig. 5) or secreted into the medium by MH_1C_1 cells.

Several lines of evidence lead us to conclude that, at the dilution of antirat serum (1:1,500) used in these experiments, the only antigen being measured by direct C′ fixation in rat serum or MH_1C_1 medium is rat albumin. C′-fixation curves obtained with purified rat albumin and whole rat serum were superimposable (Fig. 4), and the dilution of rat serum required to give peak C′ fixation agreed quantitatively with the concentration of albumin in serum. The results of experiments depicted in Figs. 4, 5, and 6 show that the antigen being measured in medium from MH_1C_1 cells is also rat serum albumin. Finally, further support for this conclusion is drawn from the results shown in Fig. 7. When increasing amounts of MH_1C_1 medium were added to the concentration of rat albumin giving peak C′ fixation, the amount of C′ fixed decreased progressively in a manner similar to that obtained with increasing amounts of rat albumin itself. When increasing amounts of rat albumin were added to the concentration of MH_1C_1 medium giving peak C′ fixation, the amount of C′ fixed decreased progressively in a manner similar to that obtained with increasing amounts of MH_1C_1 medium. If two separate immune systems were being measured (rat albumin–antirat albumin and X-antiX), MH_1C_1 medium would not substitute quantitatively for rat albumin as shown on the left in Fig. 7, and rat albumin would not substitute quantitatively for MH_1C_1 medium as shown on the right.

Results obtained by C′ fixation were verified further by double immunodiffusion (Fig. 8). A single band of precipitation was obtained with purified rat albumin. This band formed a line of coincidence without spur formation with culture medium from MH_1C_1 cells, indicating the qualitative similarity between the antigen being measured in culture medium and rat serum albumin. Whole rat serum gave multiple precipitation bands, while no reaction was seen with uninoculated medium. When concentrated antiserum was used, again only a single precipitation band formed with purified rat albumin, and a line of conicidence without spur formation was seen with medium from the hepatoma cells. However, when concentrated antiserum was used, a second precipitation band with a short spur was observed with culture medium, but not with uninoculated medium (not shown), suggesting the possibility that the MH_1C_1 cells may produce another serum

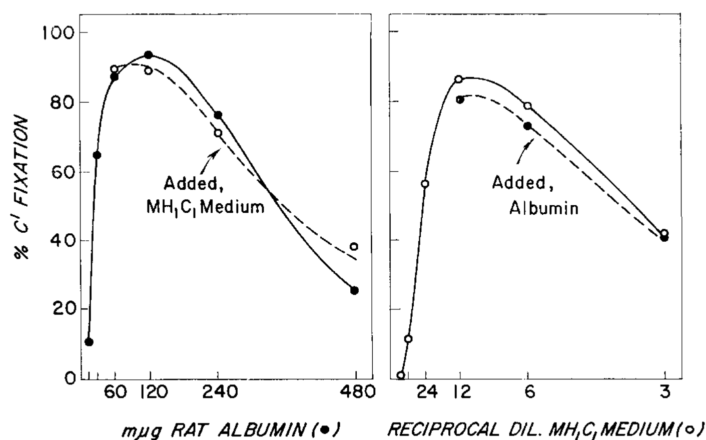

FIGURE 7 In the left panel is shown the C'-fixation curve obtained with antiserum, diluted 1:1,500, and purified rat albumin (●). A second set of reaction mixtures was made up to contain antiserum (1:1,500), a constant amount of rat albumin (60 mμg/tube), and serial (2-fold) dilutions of MH_1C_1 medium. As increasing amounts of medium from MH_1C_1 cells were added to a constant amount of rat albumin, the amount of C' fixed (○) decreased in parallel with the decrease produced by rat albumin itself (●). The reciprocal experiment is shown in the right panel. The complete C'-fixation curve was obtained with antiserum, diluted 1:1,500, and medium from MH_1C_1 cells (○). An additional set of reaction mixtures was made up to contain antiserum (1:1,500), a constant amount of MH_1C_1 medium (diluted 1:12), and serial (2-fold) increasing amounts of rat albumin. As increasing amounts of rat albumin were added to a constant amount of MH_1C_1 medium, the amount of C' fixed (●) decreased in parallel with the decrease produced by MH_1C_1 medium (○).

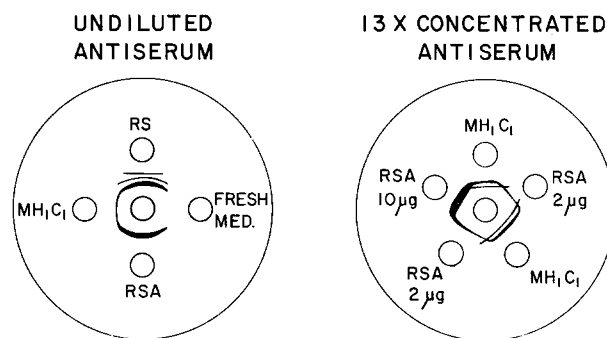

FIGURE 8 Tracing of bands of precipitation seen in agar by double diffusion for 48 hr at 4°C. The antiserum wells are in the center. Undiluted rabbit antirat serum is on the left, and 13 times (13×) concentrated antiserum is on the right. The antigen wells are whole rat serum (*RS*) diluted 1:100, fresh uninoculated medium (fresh med.), purified rat serum albumin (*RSA*), and 4-day-old medium from MH_1C_1 cells (MH_1C_1). Each well contained 0.1 ml. The amount of RSA in the well on the left was 150 μg.

protein in addition to albumin. This possibility is under further study.

Growth of Clonal Hepatoma Cell Cultures and Albumin Secretion in Vitro

MH_1C_1 cells grow slowly in culture. Shortly after initiation of the clonal strain, the cells had a population doubling time of 14 days. This time has gradually decreased over a period of 15 months, and is presently about 7 days. Fig. 9 illustrates a typical growth curve for MH_1C_1 cells studied over a period of 77 days. The logarithmic phase of cell growth extended for a period of about 5 wk and was followed by a plateau

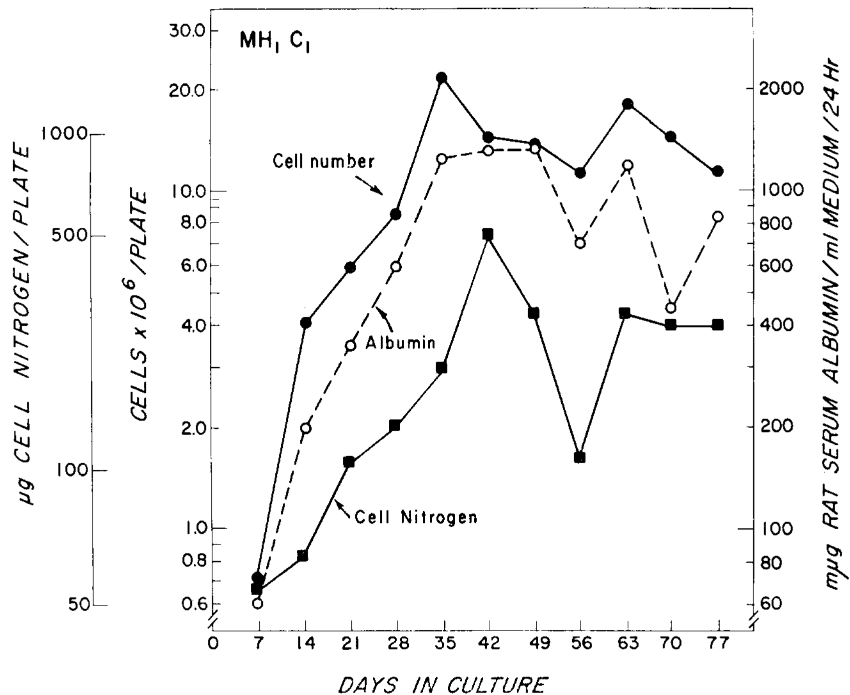

FIGURE 9 Growth curve and albumin production for cells of the MH_1C_1 strain. The closed circles (●) give the number of cells adherent to the Petri dish at weekly intervals. The closed squares (■) give the average amounts of cell nitrogen recovered from duplicate plates. The open circles (○) give the average concentrations of albumin/ml medium/day. Day zero was the day of inoculation.

phase during which approximately equal numbers of new cells were formed and old ones were shed into the medium. From the number of floating cells, it was estimated that cell proliferation slowed by a factor of approximately 2–3 during the plateau phase but did not cease. At no point was a confluent monolayer formed. Albumin was secreted into the culture medium throughout the logarithmic phase of growth. As cell growth reached the plateau phase, albumin secretion also leveled off and persisted at a rate of 500–1,000 mμg/ml of medium/24 hr (about 4–8 μg albumin/mg cell nitrogen/24 hr) for at least 50–60 days. Analyses of cells lysed by sonic oscillation revealed little or no detectable albumin inside the cells during either the logarithmic or plateau phases of cell growth. Most of the albumin appears, therefore, to be secreted into the culture medium. Similar results have been noted previously with respect to growth hormone in serially propagated rat pituitary cells in culture (5).

DISCUSSION

The present report describes a newly established strain of epithelial cells derived from the transplantable Morris hepatoma No. 7795. Immunological experiments demonstrate that the cultured hepatoma cells synthesize rat serum albumin and secrete the protein into the culture medium. The validity of this conclusion depends on the specificity of immunological experiments. Since whole rat serum was the antigen used to elicit the antiserum, antibodies to a number of components of rat serum were detected in the antiserum when it was examined by double diffusion against whole rat serum (Fig. 8). However, when tested against purified rat albumin, both undiluted and 13-fold concentrated antiserum gave only one band of precipitation, and this band gave a line of coincidence with an antigen in medium from MH_1C_1 cells. Control experiments with fresh, uninoculated medium and medium taken from other strains of rat cells (pituitary epithelial cells and

thyroid fibroblasts) gave no bands of precipitation. When this antiserum was used in quantitative C′-fixation experiments, at the dilution of 1:1,500, only one sharp C′-fixation peak was observed with whole rat serum (Fig. 4). No additional peaks of C′-fixation were seen in great antigen excess. If a contaminating antigen-antibody system were present, a second C′-fixation curve would probably have been observed (18). The single C′-fixation peak occurred with rat serum diluted 1:730,000, a dilution equivalent to approximately 65 mμg of albumin. Purified rat albumin also gave peak C′ fixation at 60–125 mμg with the same dilution of antiserum. Furthermore, the amounts of C′ fixed at antigen-antibody equivalence with rat serum and purified albumin were the same (Fig. 4). If qualitative differences were present in the 2 antigens, the amounts of C′ fixed at antigen-antibody equivalence with the two systems would be expected to be different (17).

With the same dilution of antiserum (1:1,500), medium from MH_1C_1 cells, but not fresh medium or medium from other types of rat cells in culture, gave C′-fixation curves that were similar to, but not identical with, curves obtained with purified rat albumin (Fig. 4). It was shown that the smaller (about 15%) amount of C′ fixed with medium from MH_1C_1 cells as compared to purified rat albumin was due to a minor cross-reaction of antirat albumin with horse and fetal calf albumins in the medium (Figs. 5 and 6). That rat albumin was indeed the antigen being measured in MH_1C_1 medium was verified further by the substitution and recovery experiments shown in Fig. 7.

As shown in Fig. 9, during the phase of rapid cell growth, there was a rapid increase in the concentration of albumin in the culture medium. Clearly, exponential growth of the cells did not preclude albumin biosynthesis and secretion. Albumin secretion also continued throughout a prolonged plateau phase during which the cell population density did not change appreciably. Floating cells were removed by centrifugation before the medium was assayed for albumin. They also did not contribute to the cell counts or cell nitrogen determinations. Since the ratio of floating cells to adherent cells, even late in the plateau phase, was less than 1:500–1,000, it was unlikely that they were an important source of albumin in the medium.

During the period of 15 months since the clonal albumin-secreting cells have been established in culture, there has been no evidence for prolongation of generation time or diminution of the rate of albumin synthesis, two possible indicators of cellular "senescence." Indeed, the generation time of the MH_1C_1 cell strain, though long, has decreased by 50% since the strain was first initiated.

It has previously been reported that Morris hepatoma No. 7795 has 46 or 47 chromosomes (19) as compared to the normal diploid number of 42 chromosomes in the rat. Our own analysis of the karyotype of the MH_1C_1 strain is not yet complete. However, preliminary results, obtained in collaboration with Dr. Carlos Sonnenschein, indicate 48–53 chromosomes per cultured cell.

Tomkins and his colleagues have established a different line of hepatoma cells (Morris hepatoma 7288C) in culture and have studied the induction by steroid hormones of the enzyme L-tyrosine:2-oxoglutarate aminotransferase (tyrosine aminotransferase, EC 2.6.1.5) in this cell line (20). Their extensive studies have been directed toward understanding the mechanisms of steroid-mediated induction of the enzyme and have included experiments on the roles of RNA (21, 22) and of inhibitors of nucleic acid synthesis (23) on this process. The use of hepatoma cells in culture has offered advantages in technique over studies carried out in the intact liver in vivo.

In addition to serum albumin, the MH_1C_1 cell strain also synthesizes tyrosine aminotransferase (experiments kindly performed by Doctors Brad Thompson, Gordon Tomkins, and Frank C. Bancroft). Furthermore, it responds to the addition of hydrocortisone (10^{-6} M) to the culture medium by increasing substantially the activity of the enzyme. The availability of more than one biochemical marker in the MH_1C_1 cells (serum albumin and tyrosine aminotransferase) should make this strain of mammalian cells particularly useful for studying protein synthetic control mechanisms.

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Note added in proof:

Since this manuscript was submitted for publication, tumors have developed in four out of four male Buffalo rats injected 3 months previously with cells of the MH_1C_1 strain. A primary culture was made from one of these tumors. The epithelial cells were cloned from the primary culture and were found to have the same distinct morphology as the original MH_1C_1 cell strain.

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